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design by Mirza Basic

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# HealthMED

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# Gender differences in health-related quality of life and sexual functions following percutaneous cardiovascular interventions

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**Abstract**

**Aim:** To assess health-related quality of life and sexual functions in patients who have undergone percutaneous cardiovascular intervention procedures and to determine the size and significance of gender differences.

**Methods:** The study population consisted of patients that had undergone percutaneous cardiovascular intervention procedures. The health-related quality of life was assessed with use of the World Health Organization Quality of Life Instrument, Short Form (WHOQOL-BREF) and sexual functions were evaluated by Arizona Sexual Experiences (ASEX) Scale.

**Results:** 426 patients who had undergone percutaneous cardiovascular intervention procedures met the eligibility criteria for the study and agreed to participate. Female patients had lower scores of quality of life in physical (p=0.001), psychological (p=0.001), and environmental (p=0.001) domains than male patients. Quality of life scores in social domain is not statistically significantly different between groups (p=0.725). Of the 426 patients in the study group, 234 complained of sexual dysfunction as indicated by ASEX criteria. Total ASEX scores were significantly higher in female patients (p=0.001), and the gender difference for sexual dysfunction was significant (p=0.001) for all subgroup scores.

**Conclusion:** This study provides an important complement to existing knowledge of patient outcomes after percutaneous cardiovascular intervention procedures with special emphasis on gender.

**Key words:** Percutaneous cardiovascular intervention, health-related quality of life, sexual functions, WHOQOL-BREF, Arizona Sexual Experiences Scale.

## Introduction

Since its introduction in the late 1970s, the frequency of percutaneous cardiovascular intervention (PCI) has grown substantially [1]. Subjective post-operative cognitive and emotional difficulties are very common after cardiac interventions, with self-reported depression and anxiety predicting subjective complaints [2,3]. Mood disorders, and in particular depression, are reported to be increased after PCI, although there is only a few studies that have focused on gender differences associated with the quality of life (QOL) benefits from PCI.

There has been growing interest in QOL as a part of assessment of long-term outcomes in the last few years. The World Health Organization defines QOL as 'an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns' [4]. The subjective nature of QOL means that it can be conceptualized differently by different individuals and groups of people, and is influenced by age, gender, health status and cultural factors, among others [5]. It has been demonstrated that the risk factors for cardiovascular disease influence QOL and a reduction in exposure to these risk factors would imply lengthening of life and improved health-related QOL in patients. Smoking, closely followed by high alcohol consumption and obesity are most responsible for the loss of QOL in both men and women [6]. Also, diabetes mellitus, hypertension and dyslipidaemia impact negatively on QOL in patients with cardiovascular diseases [7]. The general health-related quality of life was measured using the Quality of Life questionnaire of the World Health Organization in its shortened

version (WHOQOL-BREF) [8]. The instrument is one of the most frequently used questionnaires assessing general complaints.

Sexual dysfunction (SD) addresses alterations related to drive, subjective arousal, penile erection/vaginal lubrication, ability to reach orgasm and satisfaction with orgasm. SD and CVD appear to share pathogenic mechanisms by which traditional CVD risk factors, such as cigarette smoking, diabetes, and obesity, result in a reduction in nitric oxide activity [9]. The Arizona Sexual Experiences Scale (ASEX) is a five-item, self-administered questionnaire developed to detect and follow-up SD for depressed patients [10]. It is a quick scale to administer and requires no special training in terms of interpretation.

Numerous studies have demonstrated differences in the use of major procedures for women and men. Biases in the medical system and unmeasured clinical factors have been cited as possible explanations for these differences [11]. Although patients' attitudes are often important factors in medical decision-making, little attention has been devoted to these attitudes as potential mediators of gender differences in invasive treatments [12].

Management of patients with cardiovascular disease focuses on alleviation of symptoms, improvement in functional capacity, and alleviation of disease progression. Therefore, assessment of QOL to obtain evidence of a meaningful benefit from the patient's perspective is important. The aims of the present study were to assess QOL and sexual functions in patients who have undergone PCI procedures and to determine the size and significance of gender differences.

## Material and Methods

### *Study Design*

The current study was conducted at the Trakya University cardıology Clinic in Edirne, northwest Turkey.

The study population was composed of patients who had undergone one or more elective or urgent percutaneous coronary interventions; and patients who had undergone percutaneous pacemaker, intracardiac defibrillator or biventricular intracardiac defibrillator device implantation during the last 2 years. Patients enrolled in this study did not have any history of prior cardiovascular disease or cardiovascular intervention in the 2 years before the study. The patients with the following criteria were excluded from the study: patients who had had PCIs during the previous 2 years, those with advanced stage renal failure, dialysis therapy patients, advanced stage heart failure patients who had been frequently hospitalized, cases with congenital heart disease or bleeding diathesis who had to donate blood or receive transfusions, obese patients, cases suffering from depression,cases under 18 years of age and over 65 years of age, and cases with comorbidities. The present study was performed in cardiology outpatient clinic of the University Hospital during the period starting from January 2012 till April 2012.

The participants of the study (who had undergone PCI) were among subjects with a proper mental function, but without a life-threatening condition (requiring treatment or not) and without a previous diagnosis of any physciathric disease. After retrospective review of the medical files, 550 eligible patients meeting the criteria mentioned above were requested to participate in the study between January 2012 and April 2012. These patients were not selected according to a specific sample election technique but according to their clinical features and also volunteered to participate in the study. Of 550 patients, 426 met the criteria mentioned above for enrollment and volunteered to participate. Of the 550 candidates, 40 refused to participate in this study. During the patient interviews, clinical features of the cases were investigated and detailed again. As a result another 84 of the 550 patients were excluded because they were not eligible for our study. The eligible voluenteers, soon after their cardiac examination, were informed about the study, and were asked a couple of preliminary questions during a face to face interview in a private room. Thereafter, the questionnaire forms were filled out via asking the fundamental questions needed for the study.

Prior to beginning the research, human subject approval was obtained from the Ethical Committee of the university and from the clinic where the research was conducted; informed consent was obtained from all participants before beginning data collection.

***Outcome Parameters***

A demographic questionnaire collected data about patients' characteristics and medical status. WHOQOL-BREF questionnaire and ASEX scale were administered to all patients.

The WHOQOL-BREF is an abbreviated 26-item version of the WHOQOL-100. It contains two global items on overall QOL and general health, and four domains: Physical health domain (7 items), Psychological domain (6 items), Social relationships domain (3 items), and Environmental domain (8 items). This generates a profile of domain scores. Each item is based on a Likert scale from 1 to 5. The items ask the respondent "how much," "how often," "how completely," "how good" or "how satisfied" she felt about different aspects the respondents were asked to evaluate the potential changes in the context of a whole-time and the last 2 weeks in particular. The mean score of the items within each domain is transformed linearly to a domain score scaled in a positive direction from 4-20, such that higher scores denote higher QOL.[8] The instrument has previously been translated into our language according to existing internationally accepted guidelines, and has shown satisfactory results regarding validity and reliability [13]. In the present survey, the scale shows a good internal consistency (Cronbach α= 0.908).

Among self-rating scales for assessing sexual functioning, ASEX is used in this study. The main reason for selecting ASEX is the presence of reliability and validity studies of the Turkish version of ASEX for medical patients [14]. The ASEX is a brief 5-item scale designed to assess the core elements of sexual functioning: drive, arousal, penile erection/vaginal lubrication, ability to reach orgasm and satisfaction with orgasm. The female and male versions of ASEX differ on the gender-specific question addressing erection/lubrication. Each item is rated with a 6-point Likert system, with lower scores reflecting enhanced sexual function and higher scores reflecting impaired sexual function. A total ASEX score of 19 or greater, any one item with an individual score of either 5 or 6, or three or more items with individual scores of 4 have all been found to be highly correlated with clinician-diagnosed SD. An ASEX score of 4 or greater criterion was used for the assessment of the presence of SD of individual items. These criteria applied for the determination of SD in this study. ASEX was applied by interview to all of the subjects by the same interviewer trained in a pilot test using the same questionnaire. The subjects were alone during whole evaluation. The subjects were asked to answer the questions as they understood and the interviewer did not make any suggestions about the questions. In the present survey, the scale shows a good internal consistency (Cronbach α= 0.967).

The present study was devised as a scale-adaptation trial including subjects without a history of cardiovascular disease who had undergone PCI. In other terms, the assessment of the present status was primarily aimed. The validity and reliability of the tests used in the study have been proven and widely accepted.

***Statistical Analyses***

Data were analyzed using the Statistical Package for Social Sciences (SPSS) software (version 19.0 for Windows). A normal distribution of the quantitative data was checked using the Kolmogorov-Smirnov test. Parametric tests were applied to data of normal distribution and non-parametric tests were applied to data of questionably normal distribution. Independent-samples t-test and Mann–Whiney U-test were used to compare independent groups. Comparisons between multiple independent groups were made by Kruskal Wallis test followed by Mann-Whitney U test. To calculate correlation coefficients Pearson's r, and Kendall's tau b were used. The distribution of categorical variables in both groups was compared using Pearson chi-square, Continuity Correction, and Fisher's exact tests. Data are expressed as mean±SD or median (interquartile range), as appropriate. Statistical significance was assumed for $p<0.05$.

## Results

Four hundred and twenty-six patients met the eligibility criteria for the study and agreed to participate. Of the 426 patients, 183 (43%) were female and 243 (57%) were male, the mean age of the study group was 52.02±11.60 (range 29 to 65) years. Of the whole study group, 269 patients had undergone one or more than one percutaneous cardiovascular interventions; and 157 patients had undergone percutaneous pacemaker, or intra-

cardiac defibrillator, or biventricular intracardiac defibrillator device implantation procedure during the last two years.

As for sociodemographic and clinical characteristics of patients, 341 (80%) were married; 365 (85.7%) had at least one sexual experience in the past week; 367 (86.1%) had lower than 8 years of education; 386 (85.3%) had a partner who had lower than 8 years of education; 82 (19.2%) were employed; 164 (38.7%) were laborers; 295 (69.2%) had moderate monthly income; 367 (86.2%) were not smoking, 387 (90.8%) were not using alcohol; and 330 (91.7%) stated that they had their partner's psychological support during their treatment (Table 1).

Four hundred and twenty-one (98.8%) had a health coverage; 156 (36.6%) were living and working in the downtown for at least five years; 405 (95.1%) were regularly using drug for chronic illnesses; 345 (81%) were living in nuclear families; 215 (50.5%) were formerly treated for cardiac diseases; and 215 (50.5%) had family history for cardiac diseases. Of the female patients 166 (90.7%) were in the postmenopausal period. Among them, 17 (10%) entered menopause after hysterectomy, and 19 (11.4%) were taking hormone therapy.

The marital status (p=0.001), sexual experience (p=0.001), the level of educational (p=0.001), partner's educational level (p=0.008), employment status (p=0.001), profession (p=0.001), monthly income (p=0.059), smoking habit (p=0.001), alcohol

*Table 1. Description and comparison of demographic variables of patients according to gender*

| | | Total n (%) | Male (n=243) n (%) | Female (n=183) n (%) | p Value |
|---|---|---|---|---|---|
| Age (years) | | 62.02±11.60 (29-85) | 61.42±11.94 (36-85) | 62.81±11.11 (29-85) | 0.222 |
| Marital status | Married | 341 (80%) | 220 (90.5%) | 121 (66.1%) | 0.001 |
| | Unmarried /widowed/ divorced | 85 (20%) | 23 (9.5%) | 62 (33.9%) | |
| Sexual experience (past week) | Present | 365 (85.7%) | 230 (94.7%) | 135 (73.8%) | 0.001 |
| | Absent | 61 (14.3%) | 13 (5.3%) | 48 (26.2%) | |
| Educational level (highest level) | Primary school | 334 (78.4%) | 175 (72%) | 159 (86.9%) | 0.001 |
| | Secondary school | 33 (7.7%) | 27 (11.1%) | 6 (3.3%) | |
| | High school | 40 (9.4%) | 26 (10.7%) | 14 (7.7%) | |
| | University degree or similar | 19 (4.5%) | 15 (6.2%) | 4 (2.2%) | |
| Partner's educational level (highest level) | Primary school | 261 (76.5%) | 176 (80.0%) | 85 (70.2%) | 0.008 |
| | Secondary school | 30 (8.8%) | 11 (5.0%) | 19 (15.7%) | |
| | High school | 36 (10.6%) | 25 (11.4%) | 11 (9.1%) | |
| | University degree or similar | 14 (4.1%) | 8 (3.6%) | 6 (5.0%) | |
| Employment status | Employment | 82 (19.2%) | 72 (29.6%) | 10 (5.5%) | 0.001 |
| | Housewife /househusband/ retired | 344 (80.8%) | 171 (70.4%) | 173 (94.5%) | |
| Profession | Office holder | 68 (16.1%) | 53 (21.9%) | 15 (8.2%) | 0.001 |
| | Laborer | 164 (38.7%) | 152 (63.1%) | 12 (6.6%) | |
| | Farmer | 35 (8.3%) | 33 (13.7%) | 2 (1.1%) | |
| | Housewife | 157 (37%) | 3 (1.2%) | 154 (84.2%) | |
| Monthly income | High | 61 (14.3%) | 43 (17.7%) | 18 (9.8%) | 0.059 |
| | Moderate | 295 (69.2%) | 164 (67.5%) | 131 (71.6%) | |
| | Low | 70 (16.4%) | 36 (14.8%) | 34 (18.6%) | |
| Smoking | Absent | 367 (86.2%) | 197 (81.1%) | 170 (92.9%) | 0.001 |
| | Present | 59 (13.8%) | 46 (18.9%) | 13 (7.1%) | |
| Alcohol | Absent | 387 (90.8%) | 205 (84.4%) | 181 (99.5%) | 0.001 |
| | Present | 39 (9.2%) | 38 (15.6%) | 1 (0.5%) | |
| Partner's psychological support | Present | 330 (91.7%) | 212 (95.9%) | 118 (84.9%) | 0.001 |
| | Absent | 30 (91.7%) | 9 (4.1%) | 21 (15.1%) | |

use (p=0.001), and partner's psychological support (p=0.001) were significantly different between male and female patients (Table 1). The mean age (p=0.222); health coverage status (p=0.656); place of residence and work (p=0.787); drug use for chronic illnesses (p=0.361); family type (p=0.959); former cardiac diseases (p=0.644); and family history for cardiac diseases (p=0.341) were not significantly different between male and female patients.

The mean scores of the study group and mean scores according to gender on the different domains are shown in Table 2. Female patients had lower scores of quality of life in physical (p=0.001), psychological (p=0.001), and environmental (p=0.001) domains than male patients. Quality of life scores in social domain is not statistically significantly different between groups (p=0.725) (Figure 1). In female patients, strong correlation was determined between significantly affected QOL domains and age (r=-0.313, p=0.001), partner's age (r=-0.362, p=0.001), monthly income (r=0.268, p=0.001). Similarly, in male patients, strong correlation was determined between QOL domains and same parameters such as age (r=-0.133, p=0.003), partner's age (r=-0.185, p=0.001), monthly income (r=0.367, p=0.001).

Of 426 patients, 234 (54.9%) complained of SD as indicated by ASEX criteria. Of 234 patients, 126 (53.8%) were male, and 108 (46.2%) were female (Figure 2).

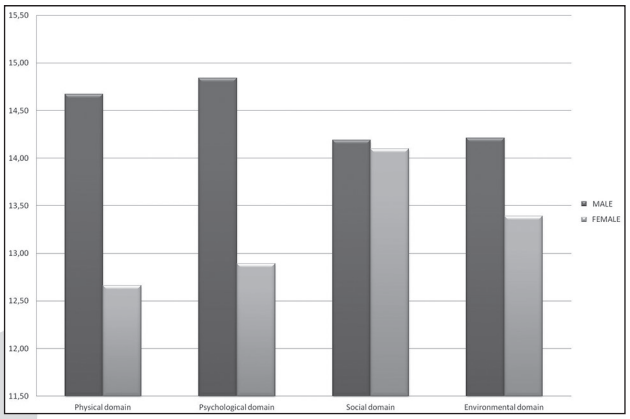


*Figure 1. The mean of each WHOQOL-BREF domain in male and female patients*

Total ASEX scores were significantly higher in female patients (p=0.001), and the gender difference for SD was significant (p=0.001). When ASEX scores of each gender were compared; ASEX-1 (sexual drive), ASEX-2 (sexual arousal), ASEX-3 (erection/lubrication), ASEX-4 (ability to reach orgasm), and ASEX-5 (satisfaction with orgasm) subscores in the female patients were found to be significantly higher than those in the male patients (Table 3) (Figure 3). Analyses of correlations between demographic variables and SD in female patients revealed significant correlations with employment status (p=001), with level of education (p=0.001), with partner's level of education (p=0.002), with profession (p=0.026), and

*Table 2. Comparison of World Health Organization Quality of Life–Brief Form scores on Physical, Psychological, Social Relationship and Environmental domains among patients according to gender*

| WHOQOL subscales | Total (mean±SD) (range) | Male (n=243) (mean±SD) (range) | Female (n=183) (mean±SD) (range) | p Value |
|---|---|---|---|---|
| Physical | 13.81±2.87 (5.71-20.00) | 14.67±2.58 (5.71-19.43) | 12.66±2.86 (5.71-20.00) | 0.001 |
| Psychological | 14.00±2.30 (5.33-20.00) | 14.84±1.90 (8.00-20.00) | 12.89±2.31 (5.33-17.33) | 0.001 |
| Social | 14.15±2.61 (5.33-21.33) | 14.19±2.34 (6.67-21.33) | 14.10±2.93 (5.33-21.33) | 0.725 |
| Environmental | 13.86±1.96 (6.00-19.50) | 14.21±1.81 (8.00-19.50) | 13.39±2.05 (6.00-19.00) | 0.001 |

*Table 3. Comparison of total and subscores of Arizona Sexual Experiences scale scores in male and female patients*

| | Male (n=243) (mean±SD) | Female (n=183) (mean±SD) | p Value |
|---|---|---|---|
| ASEX-1 (sexual drive) | 3.426±1.325 | 4.282±1.238 | 0.001 |
| ASEX-2 (sexual arousal) | 3.526±1.269 | 4.422±1.284 | 0.001 |
| ASEX-3 (erection/lubrication) | 3.735±1.296 | 4.600±1.247 | 0.001 |
| ASEX-4 (ability to reach orgasm) | 3.609±1.339 | 4.763±1.235 | 0.001 |
| ASEX-5 (satisfaction with orgasm) | 3.621±1.345 | 4.822±1.338 | 0.001 |

*SD= Standard deviation; ASEX= Arizona Sexual Experiences Scale*

with monthly income (p=0.009). In male patients SD correlated with employment status (p=001), with level of education (p=0.001), with smoking (p=0.001), with alcohol use (p=0.003), and with partner's psychological support (p=0.044). The most frequent complaints were problems with erection in male patients, while reaching and satisfaction with orgasm in female patients (Figure 3).

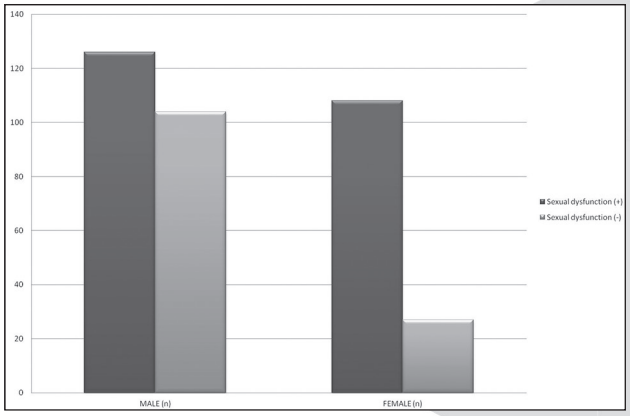


*Figure 2. Sexual dysfunction and its distribution in male and female patients*

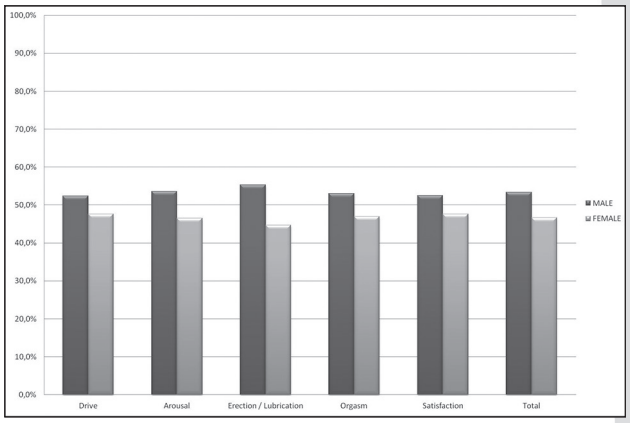


*Figure 3. Sexual dysfunction and its types in male and female patients (A total ASEX score of 19 or greater, any one item with an individual score of either 5 or 6, or three or more items with individual scores of 4 criteria were used for the assessment of the presence of SD. An ASEX score of 4 or greater criterion was used for the assessment of the presence of SD of individual items).*

## Discussion

This is one of the few studies, which investigates QOL and SD in patients who had undergone PCI. Furthermore, being the first study conducted on Turkish population, it answers the need for replicating such studies in different cultures. Our data underline that impaired QOL and SD in patients who had undergone PCI particularly occurs in female patients as worsened physical, psychological, and environmental domains in QOL; and reduced sexual drive, sexual arousal, lubrication, ability to reach orgasm, and satisfaction with orgasm.

Modern treatments, such as PCI, should focus not only on improving life expectancy, symptoms and functional status, but also on several aspects of QOL. An improvement in QOL is considered to be important as a primary outcome and in the determination of therapeutic benefit [15]. Still, results from QOL studies have not yet been brought to the forefront of this crucial discussion. One reason may be that differences in QOL scores can be difficult to interpret for clinicians and decision makers not familiar with QOL scales and line of research. A consideration of both the statistical and clinical significance of differences in QOL may prove to be valuable. Interpretation of QOL scores may also be enhanced by comparing the scores of a study population with those of a reference population. Although many investigators have described the impact of PCI on patient health status, only a few studies have examined which patient characteristics are most strongly associated with the quality-of-life benefits from PCI. In comparative studies, it has been reported that female MI survivors have physical, social, and medical disadvantages compared to their male counterparts [16,17]. Furthermore, several studies have reported lower QOL in female MI patients than in male MI patients [18,19].

In this study, we found that the men's scores were significantly higher than the women's in all four components of QOL. According to Dias *et al.*, men had a better perception of QOL than women, while women more frequently had a below-median physical score [20]. In their study, female gender was a main independent predictor of both physical and psychological components of QOL in patients with acute coronary syndrome [20]. In another study, it was indicated that women had more physical, social and psychological dysfunction, and poorer overall health than men [21]. Rankin et al. found that women had more in-hospital complications, cardiac dysfunction and mortality than men [22]. Other studies have reported that men had improved

outcomes over women [23]. However, more recent studies have reported that long-term outcomes for men and women were similar [24].

Although female patients showed significant differences on physical, psychological, and environmental health domains, overall these differences did not affect their state of mind or relationship with other people in a negative way (social domain). In the present study, female population have comparable levels of self-esteem, satisfaction with their personal relations, and social support received to those of the general female population. However, this is not true for satisfaction with sexual activity (Table 3). Although research on sexual activity following cardiac interventions is rather scarce, the existing research base suggests that PCI causes uncertainty and reduced libido [25]. Notably, most research has been focused on male erectile dysfunction; females with cardiovascular diseases have rarely been investigated.

Our data underline that SD particularly occurs in female patients as reduced sexual arousal, lubrication, ability to reach orgasm, and satisfaction with orgasm. These results are supported by previous research which reported more decrease in libido and dissatisfaction with sexual relationships in female patients compared to controls [26]. Conversely, some studies reported a higher frequency of sexual problems in male patients [27]. Possibly, in those studies, female patients were less willing to report on sexuality, or did not engage in sexual activities as frequently as men did. In our study, female interviewer might have facilitated female patients in terms of verbalizing their sexual problems or impeded male patients. Nevertheless, there are similar studies suggesting more frequent SD (like difficulties with arousal and reaching orgasm, low sexual desire and decreased libido) in female patients [26,28].

The patient group evaluated in our study consisted of patients who had had serious heart attacks or survived sudden cardiac death or suffered from serious chest pains, and those presented with heart failure or syncope, malaise or experienced severe cardiac trauma. Fear of death and feeling of distress are major complaints of these patients. In this patient group feelings, and concerns related to possible recurrence of similar complaints following intervention persist for a long time leading to deterioration of patients' quality of life. Besides, after these procedures intensive drug therapy has been instituted for these patients, and treatment-related problems such as excessive weight gain, decrease in insulin sensitivity, and diabetes, hypotension, labour loss, fatigue, and malaise, depression, insomnia, nightmares, and bleeding secondary to antiaggregant therapy might emerge [29]. The patients involving patients. After cardiovascular interventions, intense drug therapy was administered to these patients. These drugs consist of antitrombotic antiiscemic antitrombositer and antihypertension drugs. Especially the use of beta adrenerjik receptor blocking agents leads to various adverse effects such as weight gain, fatigue, and erectile dysfunction. Beta adrenergic blockers can lead to a decrease of 15% in cardiac performance. Erectile dysfunction is usually an age related adverse effect of beta adrenergic receptor blockers and was observed in 10-15% of the patients. All of these adverse effects influence the QOL unfavourably. Erectile dysfunction is also a frequently observed side effect which was seen very often in young and middle-aged men. This adverse effect mostly requires cessation of the drug therapy [30].

In addition to all these side effects, restenosis, and occlusion of the intervened vessel, and pacemaker dysfunction, chest pain, and shortness of breath restricting ambulation of the patient might cause frequent rehospitalizations. In conclusion, occasionally, procedures performed on patients and resultant prescription of intensive drug therapies might lead to the deterioration of quality of lives of the patients in the medium-, and long-term in addition to emergence of severe adverse effects with fatal outcomes.

Several potential limitations of this study should be considered. Firstly, this was a single-center study, and it is possible that unique characteristics of the patients, the physicians, or the institution will limit the generalizability of these results. Further research should examine predictors of quality-of-life improvement in larger, multicenter studies. Second limitation is the study's retrospective design leading to the absence of pre-treatment QOL scores. Third limitation is the absence of a disease-specific questionnaire. Fourth limitation is the large span of age of patients. Totally, 90.7% of the women included in the study were postmeno-

pausal, of whom 11.4% were taking hormone replacement therapy, which could affect the quality of sexual life. Besides, older people tend to have poorer quality of life.

## Conclusions

In conclusion, this study provides an important complement to existing knowledge of patient outcomes after PCI with special emphasis on gender. Furthermore, results obtained from this study provide an important step toward understanding the different effects of PCI on QOL and SD according to gender. It is hoped that this information will allow clinicians to prospectively understand the degree of postprocedural benefit patients are likely to experience, thereby supporting medical decision-making and augmenting physicians' and nurses ability to convey anticipated benefits to patients from different sexes.

## References


1. *Gibbons RJ, Chatterjee K, Daley J, Douglas JS, Fihn SD, Gardin JM, et al. ACC/AHA/ACP-ASIM guidelines for the management of patients with chronic stable angina: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on Management of Patients With Chronic Stable Angina). J Am Coll Cardiol 1999; 33: 2092-2197.*

2. *Hilborne L, Leape L, Kahan J, Park R, Kamberg C, Brook R. Percutaneous Transluminal Coronary Angioplasty: A Literature Review and Ratings of Appropriateness and Necessity. Los Angeles, Calif: RAND. 1991: 151.*

3. *Vingerhoets G. Cognitive, emotional and psychosomatic complaints and their relation to emotional status and personality following cardiac surgery. Br J Health Psychol 1998; 3: 159-169.*

4. *Organisation Mondiale de la Santé: Critères éthiques appliables à la promotion des médicaments. Genève: Organisation Mondiale de la Santé, 1998.*

5. *Nilsson J, Masudrana AM, Naharkabir Z. Social capital and quality of life in old age: Results from a cross-sectional study in rural Bangladesh. J Age Hlth 2006; 18: 419-434.*

6. *Bronnum-Hansen H, Juel K, Davidsen M, Sorensen J. Impact of selected risk factors on quality-adjusted life expectancy in Denmark. Scand J Publ Hlth 2007; 4: 1-6.*

7. *Sullivan PW, Ghushchyan V, Wyatt HR, Wu EQ, Hill JO. Impact of cardiometabolic risk factor clusters on health-related quality of life in the US. Obesity (Silver Spring) 2007; 15: 511-521.*

8. *WHO. WHOQOL User Manual. Geneva, World Health Organization; 1998.*

9. *Carrion R, Bochinski D, Rahman N, Lue T. Oral drug therapy of sexual dysfunction. In: Broderick G (eds). Physiology and Pharmacology of Erectile Dysfunction. Humana Press Inc: Totowa, NJ, 2000, pp 1–24.*

10. *McGahuey CA, Delgado LP, Geleberg AJ. Assessment of sexual dysfunction using the Arizona Sexual Experience Scale (ASEX) and implications for the treatment of depression. Psychiatric Ann 1999; 29: 39-45.*

11. *Tobin JN, Wassertheil-Smoller S, Wexler JP, Steingart RM, Budner N, Lense L, et al. Sex bias in considering coronary bypass surgery. Ann Intern Med 1987; 107: 19-25.*

12. *Ayanian JZ, Epstein AM. Attitudes about treatment of coronary heart disease among women and men presenting for exercise testing. J Gen Intern Med 1997; 12: 311-314.*

13. *Fidaner F, Fidaner C, Eser SY. [WHOQOL-100 ve WHOQOL-BREF in psikometrik özellikleri.] Article in Turkish. Psikiyatri Psikoloji Psikofarmakoloji Dergisi 1999; 7: 23-40.*

14. *Soykan A. The reliability and validity of Arizona sexual experiences scale in Turkish ESRD patients undergoing hemodialysis. Int J Impot Res 2004; 16: 531-534.*

15. *Sjöland H, Caidahl K, Wiklund I, Haglid M, Hartford M, Karlson BW, et al. Impact of coronary artery bypass grafting on various aspects of quality of life. Eur J Cardiothorac Surg 1997; 12: 612-619.*

16. *Agewall S, Berglund M, Henareh L. Reduced quality of life after myocardial infarction in women compared with men. Clin Cardiol 2004; 27: 271-274.*

17. *Kristofferzon ML, Lofmark R, Carlsson M. Myocardial infarction: gender differences in coping and social support. J Adv Nurs 2003; 44: 360-374.*

18. *Brink E, Karlson BW, Hallberg IR. Health experiences of first time myocardial infarction: factors influencing women's and men's health-related quality of life after five months. Psychology, Health and Medicine 2002; 7: 5-16.*

19. *Norris CM, Ghali WA, Galbraith PD, Graham MM, Jensen LA, Knudtson ML. Women with coronary artery disease report worse health-related quality of life outcomes compared to men. Health Qual Life Outcomes 2004; 2: 21.*

20. *Dias CC, Mateus P, Santos L, Mateus C, Sampaio F, Adão L, et al. Acute coronary syndrome and predictors of quality of life. [Article in English, Portuguese] Rev Port Cardiol 2005; 24: 819-831.*

21. *Koertge J, Weidner G, Elliott-Eller M, Scherwitz L, Merritt-Worden TA, Marlin R, et al. Improvement in medical risk factors and quality of life in women and men with coronary artery disease in the Multicenter Lifestyle Demonstration Project. Am J Cardiol 2003; 91: 1316-1322.*

22. *Rankin SH. Differences in recovery from cardiac surgery: a profile of male and female patients. Heart Lung 1990; 19: 481-485.*

23. *Higgins TL, Estafanous FG, Loop FD. Stratification of morbidity and mortality outcome by preoperative risk factors in coronary artery bypass patients. A clinical severity score. J Am Med Assoc 1992; 267: 2344-2348.*

24. *Allen JK, Xu X. Coronary revascularization in women. Crit Care Nurs Clin North Am 1997; 9: 497-509.*

25. *Steinke EE, Wright DW. The role of sexual satisfaction, age, and cardiac risk factors in the reduction of post-MI anxiety. Eur J Cardiovasc Nurs 2006; 5: 190-196.*

26. *Sakakibara R, Shinotoh H, Uchiyama T, Sakuma M, Kashiwado M, Yoshiyama M, et al. Questionnaire-based assessment of pelvic organ dysfunction in Parkinson's disease. Auton Neurosci 2001; 92: 76-85.*

27. *Macht M, Schwarz R, Ellgring H. Patterns of psychological problems in Parkinsons disease. Acta Neurologica Scandinavica 2005; 111: 95-101.*

28. *Bronner G, Royter V, Korczyn AD, Giladi N. Sexual dysfunction in Parkinson's disease. Journal of Sex and Marital Therapy 2004; 30: 95-105.*

29. *Gress TW, Nieto FJ, Shahar E, Wofford MR, Brancati FL. Hypertension and antihypertensive therapy as risk factors for type 2 diabetes mellitus. Atherosclerosis Risk in Communities Study. N Engl J Med 2000; 342: 905-912.*

30. *Brixius K, Middeke M, Lichtenthal A, Jahn E, Schwinger RH. Nitric oxide, erectile dysfunction and beta-blocker treatment (MR NOED study): benefit of nebivolol versus metoprolol in hypertensive men. Clin Exp Pharmacol Physiol 2007; 34: 327-331.*

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# Screening of soil Streptomyces and characterization of their bioactive compounds

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**Abstract**

The purpose was a study on isolation of *Streptomyces* from soil and their evaluation for production of bioactive compounds. The isolated strains with potent activity for the production were identified and verified using Api kit (bioMerieux) and 16SrRNA gene sequencing. Optimization of culture conditions was carried out at different temperatures, pHs and carbon-nitrogen sources. In addition, the growth phase of production and antimicrobial spectrum of the compounds were determined and then structure of the bioactive compounds was analyzed by nuclear magnetic resonance (NMR).

Out of 80 *Streptomyces* isolates, two strains exhibited potent activity against pathogenic bacteria. These promising strains were recognized as *Streptomyces labedae* and *Streptomyces luteogriseus*. Optimal temperature, pH and C, N-sources for the bioactive production were 25°C, 8 and glucose, maltose and yeast extract respectively. Furthermore, stationary phase was determined as the best production phase for both strains. Although *Entrobacter aerogenes*, *Bacillus cereus* and *Aspergillus niger* were sensitive *and Shigella dysenteriae, Pseudomonas aeruginosa* and *Listeria monocytogenes* were resistant to both bioactive compounds, different antimicrobial activities were observed against *Klebsiella pneumonia*, *Salmonella typhi*, *Corynebacterium glutamicum, Staphylococcus aureus* and *Candida albicans.*

1H and 13C NMR spectroscopy of the bioactive compounds exhibited straight chains with ketone, amide and alcohol groups in their structures. In general, our finding confirmed production of bioactive compound by soil *Streptomyces* and the bioactive products of these strains recommend for further investigation.

**Key words:** Streptomyces, bioactive compound, Soil.

## Introduction

The genus *Streptomyces* includes gram-positive filamentous bacteria with high level G+C content (Ningthoujam *et al.,* 2009; Shouvik *et al.,* 2012). These bacteria are aerobic and predominantly live in soil and water as microflora (Dehnad *et al.,* 2010). *Streptomyces* are usually characterized for producing secondary metabolites such as antibiotics and enzymes (Lam, 2006). Nowadays, more than 7000 compounds produced by *Streptomyces* have been identified, which most of them are secondary metabolites (Berdy, 2005).

Secondary metabolites often produce by bacteria during bacterial growth at the stationary phase. Indeed, exhaustion of a nutrient such as carbon nitrogen/phosphate and increasing the waste in the bacterial environments generate signals for production of secondary metabolites (Bibb, 2005). Biological Industry currently has focused on production of bioactive secondary metabolites and their exploitation special from soil *Streptomyces*. On the other hand, existence of high frequency of antibiotic resistant bacteria culminated in demand for the novel antimicrobial agents (Bharti *et al.,* 2010). Hence, many researchers have begun investigating on these bacteria in order to achieve new antimicrobial compounds.

Recently, isolation of bioactive producing *Streptomyces* from different geographical areas considered special field for investigation in order to obtain the new remedy for treatment of the patients. In this regard *Streptomyces* have been isolated from different areas and evaluated for production of the new antimicrobial compounds. The results obtained from their studies exhibited detection of different species of *Streptomyces* as well as various kinds of bioactive compounds(Dhanasekaran *et al.,* 2009; Dhananjeyan *et al.,* 2010; Emerson

de Lima Procópio *et al.,* 2012; Ibrahim Mabrouk, 2012). Hence, nowadays several talents have been done to introduce the new bioactive compounds in order to eliminate the rate of antibiotic resistant bacteria. The present study was undertaken to survey on isolation of soil origin *Streptomyces* from our geographical areas and the evaluation of their antimicrobial compounds.

## Materials and methods

### *Sample collection and pretreatment*

In total, 300 soil samples were collected from various areas in the north, center and south of Iran. Each sample was taken from profoundness of 10-15 cm. Then the samples were air-dried and prepared. For isolation of *Streptomyces,* one gram of each prepared soils was dissolved in 99 ml sterile distilled water and kept in an shaker at 150 rpm for 30 min. Then, the samples were full cultured on the Trypticase soy agar plates and incubated at 28-30$^0$ C for 5-6 days (Ningthoujam *et al.,* 2009).

### *Screening and Identification of bioactive Streptomyces*

Preliminary identification of *Streptomyces* isolates was carried out by the colony characteristics, type of areal hyphae, growth of vegetative hyphae, fragmentation pattern and spore formation. After the initial identification of the isolated *Streptomyces*, productions of antimicrobial metabolites were evaluated by using the Agar Well Diffusion method. Each pure sample was cultured in TSB and shaked in an orbital shaker at 25-30$^o$C with 150 rpm for 48-72 h. The broth was centrifuged at 10,000 rpm for 10 min and filtered by Whatman No.1 paper. The antimicrobial activity of the filtrate was assayed against *Escherichia coli* (PTCC 1330), *Pseudomonas aeruginosa* (PTCC 1074), *Bacillus cereus* (PTCC 1015)*, Staphylococcus aureus* (PTCC 1112) and *Aspergillus niger* (PTCC 5012) using Muller Hinton Agar (MHA) (Vimal *et al.,* 2009; Bharti *et al.,* 2010).

To perform the experiment each bacterium was fully cultured on MHA, then wells were made in the medium using sharp borer. Afterward 100 µl of the supernatant was added to each well and the plates were incubated at 37$^o$C for 24 h. Subsequently a zone of inhibiting growth considered the antimicrobial effect of the metabolite and the size was measured and recorded (Voravuthikunchai *et al.,* 2006).

### *Identification and authentication of bioactive producing Streptomyces*

Out of five *Streptomyces* isolates, two strains showed potent activity in production of antimicrobial metabolites. These strains were subjected for phenotypic and molecular identification. Phenotypic identification was carried out using catalase, oxidase, nitrate, oxidative / fermentative tests and APi coryne kit (bioMerieux). In addition authentication of the bioactive strains was done by 16SrRNA gene DNA sequencing. To perform the experiment DNA extraction was carried out using DNA PCR kit (Roche-Germany). Then the purity of extracted DNA was assessed by absorbance at 260 and 280 nm. The extracted DNA with ratio (260/280nm) of 1.9≤ corresponding to 121 µg DNA ml $^{-1}$ was used for Polymerase Chain Reaction (PCR). Amplification of 16SrRNA gene was performed using Forward and Reverse primers with sequences of 5'-CAACGAGCG-CAACCCT-3' and 5'- GGTTACCTTGTTAC-GACTT -3' respectively. Each reaction tube was containing 14.5 µl of water (Sigma Aldrich Company Ltd.), 2.5 µl of 10×PCR buffer (Cinagen-Iran), 1 µl of each forward and reverse PCR primers, 1 µl of a 10 mM dNTPs (Cinagen-Iran), 0.5 µl of Smar taq polymerase (cinagen-Iran), 1 µl of 50mM $MgCl_2$ (cinagen-Iran) and 5 µl of DNA template. PCR conditions of thermocycler (Cleaver, England) were as follows: 95°C for 3 min, followed by 35 cycles of 95°C for 60 s, 56°C for 45 s, and 72°C for 60 s, with a final extension at 72°C for 5 min and storage at 4°C. All the PCR products were run on a 1.5% (w/v) agarose gel. PCR products were electrophoresed at 75V for 20 min and then DNA bands were virtualized after staining with ethidium bromide. Finally the PCR products with pure DNA bands have been sent to Macrogen in South Korea (http://www.macrogen.com/) for DNA sequencing. The 16S rRNA sequenced data were subjected to BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST/) to identify each respective 16S rRNA gene amplicon.

***Separation of bioactive compounds by different solvents***

The experiment was carried out by cultivation of the strains in Trypticase soy broth and incubated in a shaker incubator at 150 rpm at 25-30°C for 48-72 h. After three days the broth was centrifuged at 10,000 rpm for 10 min. Then the mycelia biomass was separated by filter paper Whatman No.1 (Bharti *et al.,* 2010). The extraction of antimicrobial metabolites was done using different solvents viz., ethyl acetate, chloroform, acetone and ethanol. The solvents separately were added to the filtered supernatants in 1:1 proportion, and mixed by homogenizer for 45 min. The solvents were centrifuged at 5000 rpm for 15 min and the solvent parts were evaporated at 70 and 80 $^0$C after separation of their aqueous phase. The dark, brown and gummy compounds that obtained were subjected for determination of antimicrobial activities (Augustine *et al.,* 2005; Dehnad *et al.,* 2010).

***Culture Optimization***

Culture optimization for maximum production of antimicrobial metabolites was carried out by changing temperatures, pHs, carbon and nitrogen sources.

To perform the tests, isolated *Streptomyces* were inoculated into the TSB and incubated at temperatures of 25, 30, 35 and 40° C for 72 h. Then 100 µl of each supernatant (centrifugation at 10000rpm, 20 minutes) was added into the wells on the Muller Hinton agar seeded by *Bacillus cereus* (PTCC1015), and incubated at 37° C. After 24h, the effect of temperatures on bioactive compound production was determined based on the size of inhibition growth zone.

To determine the best functional pH, the pH of TSB was adjusted at 5, 6, 7 and 8. Then the bacteria were separately inoculated and incubated at 27° C. After 72h, 100 µl of each supernatant (centrifugation at 10000rpm, 20 minutes) was added into the wells on the Muller Hinton agar seeded by with *Bacillus cereus* PTCC1015 and incubated at 37° C. After 24h, the effect of pH on bioactive compound production was determined based on the size of inhibition growth zone.

To determine the best carbon and nitrogen sources glucose, lactose, maltose, sucrose, fructose, starch, glycerol, peptone, yeast extract and trypton were separately sterilized and added into the TSB with 1% concentration. Then, *Streptomyces* isolates were inoculated to the medium and incubated in shaker incubator at 150 rpm, 25-30$^0$C for 48-72 h. Then handy carbon and nitrogen sources were selected on the basis of the inhibition growth zone, as mentioned above (Saurav and Kannabiran, 2010)

***Arbitrary Units (AU) of bioactive compounds***

To determine AU, various dilutions $1^{-2}$, $1^{-4}$, $1^{-8}$, $1^{-16}$, $1^{-32}$, $1^{-64}$, $1^{-128}$ and $1^{-256}$ were made from each bioactive compound. Then 100 µl of each dilution was poured into the well the wells on the Muller Hinton agar seeded by with *Bacillus cereus* PTCC1015. The plates were incubated at 37 °C for 24 hrs. Arbitrary Unit of each metabolite was determined by reciprocal of highest dilution exhibiting antimicrobial effect (Voravuthikunchai *et al.,* 2006).

***Determination of the phase of bioactive compounds production***

To obtain the phase of bioactive compound production, the strains separately inoculated in TSB and incubated in a shaker incubator at 150 rpm 25-30°C, for 48-72 h. Then optical density (OD) of the broth was determined at 620 nm every 12 h. At the same time antimicrobial activities of each sample was determined as explained above (Moshafi *et al.,* 2011).

***Determination of Antimicrbial Spectrum of metabolites against pathogenic microbes***

Antimicrobial spectrum of bioactive compounds was assessed against pathogenic organisms viz., *Escherichia coli*(PTCC 1330), *Entrobacter aerogenes* (PTCC 1221), *Pseudomonas aeruginosa*(PTCC 1074), *Klebsiella pneumoniae*(PTCC 1053), *Bacillus cereus*(PTCC 1015), *Listeria monocytogenes* (PTCC 1298), *Salmonella typhi*(PTCC 1609), *Shigella dysenteriae*( PTCC 1188), *Corynebacterium glutamicum (*PTCC 1532), *Staphylococcus aureus*(PTCC 1112), *Candida albicans*(PTCC 5027), *Aspergillus niger*(PTCC 5012), *Cladosporium sp.*(PTCC 5202), *Penicillium sp.*(PTCC 5251), *Rhizopus oryzae*(PTCC 5174) and *Mocur hiemalis*(PTCC 5292) were used. To perform the test, each bioassay strain was fully cultivated on Mueller Hinton agar and two wells were

made in the plate agar using sterile sharp borer. Then, 100 µl of purified antimicrobial metabolites were separately added into each well and the plate was incubated at 37°C. After 24h, the size of inhibition growth zone was measured and considered antimicrobial activity of the compound. All experiments were carried out in three replicates.

### *Nuclear magnetic resonance analysis of the metabolites*

Three and ten milliliters of the pure antimicrobial metabolites were subjected for 1HNMR and 13CNMR (300 MHz, Brucker Biospin, Switzerland). The metabolites dissolved in 3 ml of Acetone and analyzed by nuclear magnetic resonance (NMR) (Augustine *et al.,* 2005).

## Results

### *Isolation of bioactive Streptomyces*

In Total 80 strains of *Streptomyces* were isolated and assessed for the production of bioactive compounds. Out of all, two strains could produce compounds and tested against bioassay strains of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus* and *Aspergillus niger*. As showed in Table 2 *Bacillus cereus* and *Aspergillus niger* were sensitive to both compounds, however *Pseudomonas aeruginosa* was resistant to them. Furthermore, different responses of compounds were observed against *Escherichia coli* and *Staphylococcus aureus. Escherichia coli* and *Staphylococcus aureus* were sensitive to the metabolites produced by isolated strains of No. 1 and No. 2 respectively.

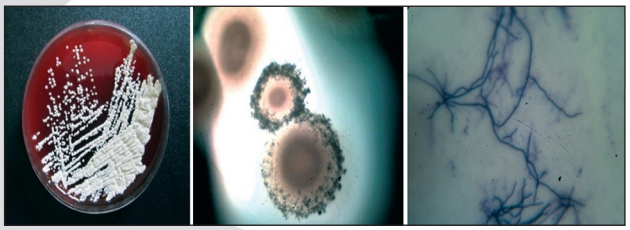
*Table 1. Antimicrobial activity of the compounds produced by Streptomyces isolates*

| Inhibition Zone Diameter (mm) of metabolites | | |
|---|---|---|
| **Microorganisms** | **Produced by strains** | |
| | **No1** | **No2** |
| *Escherichia coli* | 10 | -* |
| *Pseudomonas aeruginosa* | - | - |
| *Staphylococcus aureus* | - | 20 |
| *Bacillus cereus* | 14 | 15 |
| *Aspergillus niger* | 14 | 12 |

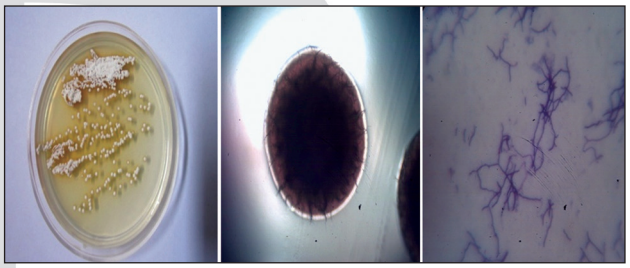
**, no zone*

### *Identification of bioactive producing Streptomyces*

The results obtained from phenotypic identification of the isolates illustrated that antimicrobial producing bacteria were *Streptomyces* spp. However, Alignment analysis of 16SrRNA genes of the bacterial strains exhibited 94% and 98% identical to *Streptomyces labedae* (No1)with accession number gb|JQ738392.1| and *Streptomyces luteogriseus* (No2)with accession number gb|GQ985454.1|respectively. Colony characters and the cell properties of *Streptomyces* isolates are shown in plate 1.



*No.1*



*No.2*

*Figure 1. Macroscopic and microscopic properties of bioactive producing Streptomyces isolates.*

### *Extraction of the bioactive compounds by different solvents*

The result obtained from extraction of the bioactive compounds by different solvents indicated that out of all solvents acetone follow by ethyl acetate, chloroform and ethanol were respectively the best solvents for extraction of the compounds produced by *Streptomyces labedae* and *Streptomyces luteogriseus.*

### *Optimization of bioactive compounds production*

The results obtained from optimization of bioactive compound production by the isolated strains showed that the best temperature and pH for the production was 25 °C and neutral to slightly alkaline for both strains. However maltose and

yeast extract and glucose, maltose and yeast extract were preferred for the production of bioactive compounds by *Streptomyces labedae*, and *Streptomyces luteogriseus* respectively (Tables 3 and 4). Arbitrary Units obtained for both bioactive compounds were 128 AU.

***Production of bioactive compounds at different growth phases of Streptomyces isolates***

The results obtained from the production of bioactive compounds during growth of *Streptomyces labedae* and *Streptomyces luteogriseus* indicated that the production of the compounds was started on 60th hours and reach to maximum level on 84th of the bacterial growth. Hence, stationary phase considered a phase of production and the compounds as secondary metabolites (Figure 1).

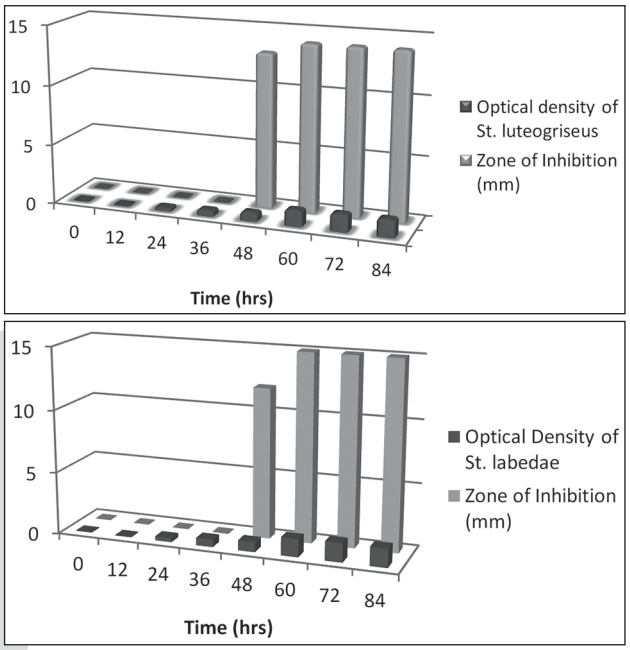



*Figure 2. Growth curves and its relations with antimicrobial metabolites production*

*Table 2. Effect of different pHs and temperatures on the bioactive compound production by Streptomyces isolates*

| Inhibition zone diameter (mm) of metabolites at | | | | | | | |
|---|---|---|---|---|---|---|---|
| Isolated Strains | pHs | | | | Temperature | | |
| | 5 | 6 | 7 | 8 | 25 | 30 | 35 |
| *Streptomyces labedae* | - * | 10 | 14 | 15 | 14 | 15 | - |
| *Streptomyces luteogriseus* | - | 11 | 15 | 15 | 15 | 15 | - |

**, no zone*

*Table 3. Effect of different carbon and nitrogen sources on the bioactive compound production by Streptomyces isolates*

| Inhibition zone diameter (mm) of metabolites in present of | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolated Strains | Carbon | | | | | Nitrogen | | | | | |
| | glucose | lactose | maltose | sucrose | fructose | starch | glycerol | peptone | yeast | extract | trypton |
| *Streptomyces labedae* | 16 | 15 | 17 | 16 | 14 | 16 | 14 | 14 | 17 | 14 | |
| *Streptomyces luteogriseus* | 19 | 17 | 19 | 18 | 16 | 16 | 16 | 17 | 19 | 15 | |

*Table 4. Antimicrobial spectrum of the metabolites produced by Streptomyces against pathogenic microorganisms*

| inhibition zone diameter (mm) of metabolites produced by | | |
|---|---|---|
| Pathogenic microorgamisms | Streptomyces labedae | Streptomyces luteogriseu |
| *Entrobacter aerogenes* | 12 | 10 |
| *Klebsiella pneumonia* | *- | 10 |
| *Salmonella typhi* | - | 12 |
| *Shigella dysenteriae* | - | - |
| *Listeria monocytogenes* | - | - |
| *Corynebacterium glutamicum* | - | 11 |
| *Candida albicans* | 17 | - |

**, Resistant*

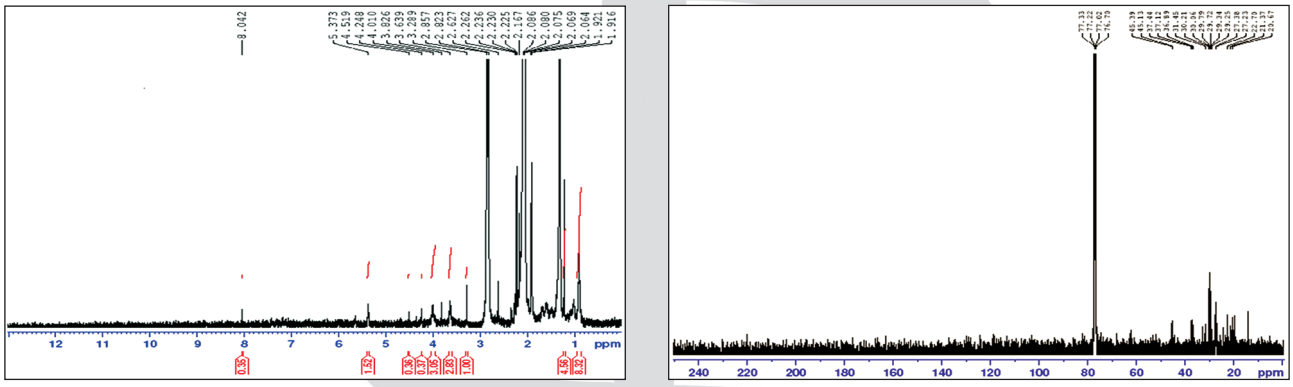
***Antimicrbial Spectrum of the bioactive compounds against pathogenic microbes***

The result obtained from antimicrobial spectrum of the metabolites produced by *Streptomyces labedae* and *Streptomyces luteogriseus* illustrated that *Entrobacter aerogenes* was sensitive and *Listeria monocytogenes* and *Shigella dysenteriae* were resistant to the both compounds. In addition, *Candida albicans* and *Corynebacterium glutamicum, Klebsiella pneumoniae* and *Salmonella typhi* showed sensitive character to the bioactive compounds produced by *Streptomyces labedae* and *Streptomyces luteogriseus* respectively (table 5).

***Structural analysis of the bioactive compounds produced by Streptomyces labedae and Streptomyces luteogriseus***

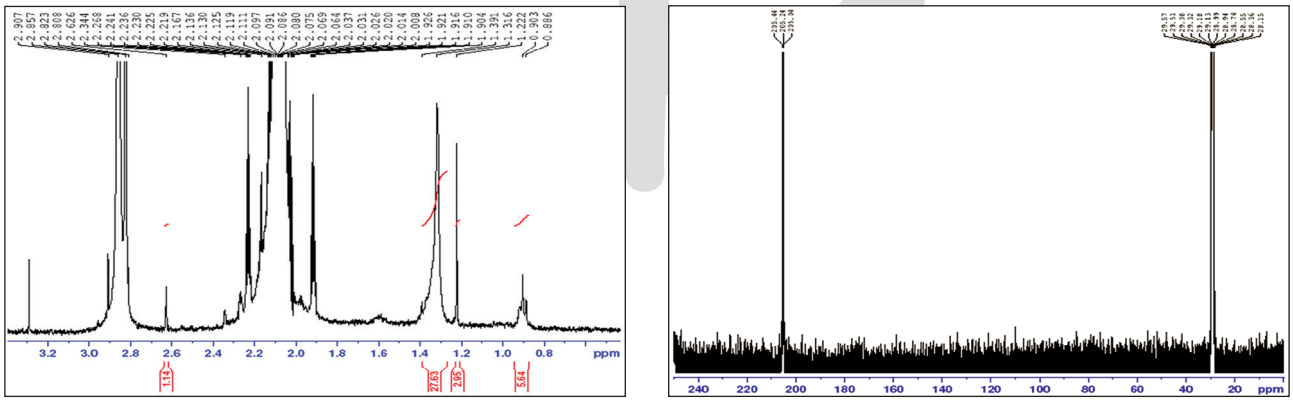
1H NMR (500 MHz) spectrum of the bioactive compound produced by *Streptomyces labedae* in acetone has large peaks in the regions 1,3, 2.08, 2.82 and small peaks in regions 0.9 and 3 to 8. Major peaks probably indicates existence of HO-CH- and –C= CH- protons in the structure of the compound. Regarding to The 13C NMR (300 MHz) showed -C-NO2 at region 80 and -C=C- group at regions 15 to 45 ppm. Therefore, based on foregoing data formula of the compound probably is C12H19O5N2 with an amide and two alcohol groups.

The results obtained from 1H NMR spectrum of the bioactive compound produced by *Streptomyces luteogriseus* in acetone indicated that large peaks were observed in regions 2.08, 2.09 and 2.8, 2.9. small peaks were observed in regions 0.9, 1.2, 1.3, 2.6 and 2.9. Major peaks probably indicates –C= CH- , HO-CH- and CH-OR protons. 13C NMR showed two major peaks in regions 29 and 205 ppm and probably indicates -C-NO2 and R2C=O respectively Therefore, based on foregoing data formula of the compound probably is C11H14O4N with one Keton and three double bonds groups (Figure 2 and 3).



*(a)* *(b)*

*Figure 3. 1H (a)and 13C (b)NMR (300 MHz) spectrum of the metabolite produced by Streptomyces labedae*



*(a)* *(b)*

*Figure 4. 1H (a) and 13C (b) NMR (500 MHz) spectrum of the metabolite produced by Streptomyces luteogriseus*

## Discussion

Nowadays several antibiotics have been recommended for decreasing the rate of occurrence of antibiotic-resistant bacteria. In this regard pharmaceutical industries made attempts to introduce the new sources of antimicrobial components with potent activity against pathogenic bacteria, viruses and etc (Dehnad *et al.,* 2010; Mageshwaran *et al.,* 2011; Gontang *et al.,* 2007). Successful discoveries in this area were shown a reasonable strategy for the isolation and characterization of bacteria with high level of bioactive compound production (Basik *et al.,* 2003). Out of all bacteria, *Streptomyces* were a special target bacteria in drug industries because of their ability to produce a variety of bioactive compounds (Eccleston *et al.,* 2008; Watanabe *et al.,* 2003). Many reports confirmed isolation of the bioactive compound producing strains belonging to the genus *Streptomyces* from Thailand , Jordan, Egypt and (Bull *et al.,* 2000; Saadoun and Gharaibeh, 2003). Although *Streptomyces* have been recognized as the potential antibiotic producer bacteria, the type of their bioactive compounds could be depended on the environment factors and the geographical areas. For instance, *Streptomyces* isolates from Australian coastal zone could produce aromatic polycyclic compounds with antibacterial activity against gram-positive and negative bacteria (Eccleston *et al.,* 2008). Several investigates in India, Africa and Myanmar exhibiting different antimicrobial spectrum of the bioactive compounds produced by *Streptomyces* isolates against pathogenic bacteria (Ningthoujam *et al.,* 2009).

The results obtained from our study indicated that out of 80 strains of indigenous *Streptomyces*, two strains had potent activity for production of bioactive compounds. Phenotypic and molecular identification of the isolates recognized them as *Streptomyces labedae* and *Streptomyces luteogriseus*. Optimal temperature, pH and C, N-sources for growth of these strains were 25°C, 8 and glucose, maltose and yeast extract respectively. Concerning to the growth phase of production, stationary phase was recognized as a major the production phase and acetone was a superior solvent. Therefore based on foregoing evidence the bioactive compounds produced by these bacteria were secondary metabolites and considered as antibiotics.

In addition 1H and 13C NMR analysis of the bioactive compounds exhibited straight chains with different groups of ketone, amide and alcohol. However varied responses of the compounds against pathogenic microorganisms might be related to different chemical groups, which are linked to their straight chains.

## Acknowledgements

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## References

1. *Augustine SK, Bhavsar SP, Kapadnis BP. A non-polyene antifungal antibiotic from Streptomyces albidoflavus PU 23. Indian Academy of Sciences., 2005; 30: 201–211.*

2. *Baserisalehi M, Bahador N. Isolation and molecular identification of Pseudomonas Spp. from soil samples and their evaluation for bacteriocin production. Asian Pacific Journal of Tropical Biomedicine.,2012; 1-5.*

3. *Basik, M, Mousses S, Trent J. Integration of genomic technologies for accelerated cancer drug development. Biotechniques., 2003; 35: 580-586.*

4. *Berdy J. Bioactive microbial metabolites - A personal view. J Antibiot, 2005; 58: 1-26.*

5. *Bharti A, Kumar V, Gusain O, Singh Bisht G. Antifungal activity of Actinomycetes isolated from Garhwal region. Journal of Science Engg. & Tech. Mgt. 2010; 2: 3-9.*

6. *Bibb MJ. Regulation of secondary metabolism in streptomycetes. Curr Opin Microbiol. 2005; 2: 208-215.*

7. *Bull AT, Ward AC, Goodfellow M. Search and discovery strategies for biotechnology: The paradigm shift. Microbiology and Molecular Biology., 2000; 64: 573-606.*

8. *Dehnad AR, Parsa YR, Bakhshi R, Mokhtarzadeh A, Soofiani SA, Monadi AR et al. Investigation antibacterial activity of Streptomycetes isolates from soil samples, West of Iran. African Journal of Microbiology Research., 2010; 4: 1685-1693.*

9. *Dhananjeyan V, Selvan N, Dhanapal K. Isolation, characterization, screening and antibiotic sensitivity of Actinomycetes from locally (Near MCAS) collected soil samples. J. Biol. Sci., 2010; 10: 514-519.*

10. *Dhanasekaran D, Selvamani S, Panneerselvam A, Thajuddin N. Isolation and characterization of Actinomycetes in vellar estuay, annagkoil, tamil nadu. African Journal of Biotechnology., 2009; 8: 4159-4162.*

11. *Eccleston GP, Brooks PR, Kurtböke DI. The occurrence of bioactive Micromonosporae in aquatic habitats of the Sunshine coast in Australia. faculty of science, health and education, university of the Sunshine coast, Maroochydore DC, Qld 4558, Australia., 2008; 6: 243-261.*

12. *Emerson de Lima Procópio R, Reis da Silva I, Kassawara Martins M, Lúcio de Azevedo J, Magali de Araújo J. Antibiotics produced by Streptomyces. The Brazilian Journal of Infectious Diseases., 2012; 16 5: 466-471.*

13. *Gontang EA, Fenical W, Jensen PR. Phylogenetic diversity of gram-positive bacteria cultured from marine sediments. Applied Environment Microbiology., 2007; 73: 3272-3282.*

14. *Ibrahim Mabrouk M. Investigation on some Streptomyces species produce antibiotic with immobilized cells by using calcium alginate. Journal of Applied Sciences Research., 2012; 8: 1466-1476.*

15. *Lam KS. Discovery of novel metabolites from marine Actinomycetes. Current Opinion of Microbiology., 2006; 9: 245-251.*

16. *Mageshwaran V, Walia S, Govindasamy V. Antibacterial activity of metabolite produced by Paenibacillus polymyxa strain HKA-15 against Xanthomonas campestris pv. Phaseoli. Indian Journal of Experimental Biology., 2011; 49: 229-233.*

17. *Moshafi MH, Forootanfar H, Ameri A, Shakibaie M, Dehghan-noudeh G, Rzavi M. Antimicrobial Activity of Bacillus sp. Strain FAS1 Isolated From Soil. Pakistan Journal of Pharmacology Sciences., 2011; 24: 269-275.*

18. *Nanjwade BK, Chandrashekhara S, Shamarez AM, Goudanavar PS, Manvi FV. Isolation and morphological characterization of antibiotic producing Actinomycetes. Tropical Journal of Pharmaceutical Research., 2010; 9: 231-236.*

19. *Ningthoujam DS, Sanasam S, Nimaichand S. Screening of Actinomycete isolates from niche habitats in manipur for antibiotic activity. Microbial Biotechnology Research Laboratory, Department of Biochemistry, Manipur University, Canchipur, Imphal. American Journal of Biotechnology., 2009; 5: 221-225.*

20. *Saadoun I, Gharaibeh R. The Streptomyces flora of Badia region of Jordan and its potential as a source of antibiotics active against antibiotic-resistant bacteria. Journal of Arid Environ., 2003; 53: 365-371.*

21. *Saurav K, Kannabiran K. Diversity and Optimization of process parameters for the growth of Streptomyces VITSVK9 spp. isolated from Bay of Bengal, India. Journal of Natural & Environmental Sciences., 2010; 1: 56-65.*

22. *Shouvik S, Pranab R, Sutanu S. Actinomycetes from hospital dump soil produce highly activity antibiotic. International Journal of Microbiology Research., 2012; 4: 258.*

23. *Vimal V, Mercy Rajan B, Kannabiran K. Antimicrobial Actvity of Marine Actinomycete, Nocardiopsis sp. VITSVK 5 (FJ973467). Asian Journal of Medical Sciences., 2009; 1: 57-63.*

24. *Voravuthikunchai SP, Bilasoi S, Supamala O. Antagonistic activity against pathogenic bacteria by human vaginal lactobacilli. Anaerobe., 2006; 12: 221–226.*

25. *Watanabe Y, Shinzato N, Fukatsu T. Isolation of Actinomycetes from termites' guts. Bioscience Biotechnology and Biochemistry., 2003, 67: 1797-1801.*

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# The comparison of arterial stiffness among patients treated with hemodialysis or peritoneal dialysis

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**Abstract**

**Background:** Arterial stiffness which is an indicator of atherosclerosis is an important risk factor for cardiovascular morbidity and mortality. End stage renal disease (ESRD) increases arterial stiffness. We aimed to compare arterial stiffness among ESRD patients treated with hemodialysis (HD) or peritoneal dialysis (PD).

**Method:** The study included 55 patients treated with hemodialysis (n: 25) and peritoneal dialysis (n: 30). Arterial stiffness was determined by aortic strain, dystensibility and aorta-femoral pulse-wave propagation velocity.

**Results**: Diabetes mellitus (%32 versus 10%) and hypertension (%60 vs %36.7) were found significantly more frequent in HD patients. However total cholesterol (203.9 ± 59.3 versus 162.0 ± 43.7; p=0.005), low-density lipoprotein (118.3 ± 38.9 versus 95.4 ± 31.1; p=0.021) and triglyceride (258.7 ± 200.8 versus 174.2 ± 76.0; p=0.050) levels were significantly higher in PD patients. Aortic strain (8.72 ± 4.17 versus 9.65 ± 5.54; p=0.478), dystensibility (0.39 ± 0.21 versus 0.45 ± 0.26; p=0.329) and PWPV (13.7 ± 0.41 versus 12.9±0.47; p=0.512) were not significantly different between HD and PD patients.

**Conclusion:** There was no significant difference between HD and PD patients in view of arterial stiffness.

**Key words:** Arterial stiffness, atherosclerosis, hemodialysis, peritoneal dialysis.

## Introduction

Cardiovascular diseases are the major cause of morbidity and mortality in patients with End-stage renal disease (ESRD) [1]. The increase in cardiovascular disease (CVD) among ESRD patients is not related only with traditional risk factors, but also can be related with conditions such as hypertension dependent to chronic hypervolemia, anemia, of the calcium-phosphor metabolism, hypercatabolism, hyper homocysteinemia and chronic micro-inflammatory disorder related with increased oxidative stress which are characteristic for uremia[2]. The cardiovascular diseases mentioned herein which are considered as the reasons of morbidity and mortality in ESRD patients are related with atherosclerosis [3].

Early diagnosis of atherosclerosis is possible after assessing the mechanical characteristics of the aorta by non-invasive methods. Aortal stiffness which is recognized as an indicator of atherosclerosis is an important risk factor for cardiovascular morbidity and mortality [4]. An increase in aortal stiffness may cause an increase in systolic blood pressure, ventricular after load and pulse pressure and a decrease in subendocardial blood flow and an escalation of the pulsatile stress in peripheral arteries [5]. Aortal stiffness can be effected due to physiological conditions such as age, gender, body weight, hormonal status and genetic characteristics and environmental factors such as nutrition, cigarette smoking and exercising capacity, and certain conditions such as hypertension, hypercholesterolemia, diabetes mellitus, coronary arterial disease, cerebrovascular disease and renal failure [6].

In some studies (7-9), arterial stiffness has increased in PD patients more than HD patients. Moreover, there is also the opposite findings (10). In our study, there was no significant difference between HD and PD patients in view of arterial stiffness.

Our purpose in this study is to compare arterial stiffness by using aortic strain, dystensibility and pulse-wave propagation velocity (PWPV) parameters in patients who are treated with hemodialysis (HD) and peritoneal dialysis (PD).

## Material and method

After a permit was obtained from the Yuzuncu Yil University, Faculty of Medicine, Committee of Scientific Research and Ethics we carried out the current study between January 2009 and July 2010, on hemodialysis and peritoneal dialysis patients who were followed up by the Yuzuncu Yil University, Faculty of Medicine, Scientific Department of Nephrology. A total number of 55 voluntary patients treated with HD (n: 25) and PD (n: 30) were included into the study.

Patients who undergone HD or PD for a period less than 6 months, under 18 years of age, with cardiac failure, malignancy, communication problems and who refused to participate in the study.

Physical examination was carried out after patients were interviewed and demographical data were recorded. After then, patients were assessed by 12 derivation electrocardiography (ECG) and transthoracic echocardiography (TTE) (Vivid 3, General Electric, 3-MHz transducer).

Entire individuals were studied at a left lateral decubit position and echocardiograms were recorded as standard parasternal and apical axis. Left atrium diameter, systolic and diastolic aortic diameters, left ventricular cavity systolic and diastolic dimensions, interventricular septum and posterior wall thickness were determined by a parasternal long axis M-mode method. All measurements were obtained by considering the basic principles of the Association of the American Echocardiography Standards [11].

Aortic strain, dystensibility and PWPV were employed as arterial stiffness parameters.

Aortic strain (%) = (systolic aortic diameter - diastolic aortic diameter) x 100 / diastolic aortic diameter (12).

Aortic dystensibility ($cm^2/dyn/10^3$) = (2 × aortic strain) / (pulse pressure) [12,13]

Pulse-wave propagation velocity (m/sn) = Patients were pre-emptively monitored by a 3-leads ECG. After then, thoracic aorta was targeted from the descendant at the subclavian artery outlet alignment by inserting the 3 MHz TTE probe to the suprasternal window while the patient laid in a supine position.

The left common femoral artery was used as the distal measurement point (Figure 1). Systolic deflexion baselines in Doppler spectral recording which were obtained by a continuous flow Doppler, received from aortic and femoral arteries were used as a point of reference and the difference between distances with the R wave in ECG recordings are recorded as the period between pulse-wave propagation velocity reference points (transit period (T)). Thereafter the distance between superficial measurement and the reference point (D) was determined as a meter value [14]. Accordingly; PWPV (m/sn) = D / T

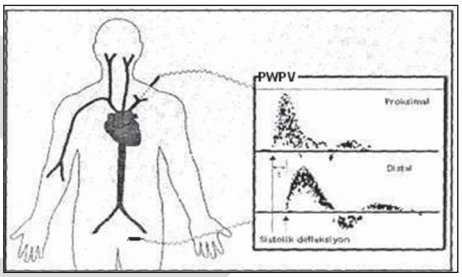


*Figure 1. Aortic arch and left common femoral artery were used as a point of reference and the difference between distances with the R wave in ECG recordings are recorded as the period between pulse-wave propagation velocity reference points*

### *Statistical analysis*

Continuous variables are stated as numbers and percent values of categorical variables, likewise Average, Standard Deviation, Minimum and Maximum Values. Group averages of continuous variables were compared by the aid of a student-t test. The relationship between these variables were assessed by the means of a Pearson Correlation Analysis. However, a Q-Square Test was used to compare categorical variables. Statistical significance level was considered as $p<0,05$ and a SPSS 16.0 statistic package program was used during calculations.

## Results

The mean renal replacement period (RRP) of hemodialysis patients was 45.40 ± 28.76 months (6

– 120 months) while the this period in PD patients was 49.60 ± 25.35 months (12 – 120 months). No any statistically significant difference was found regarding age, RRP, weight, pulse rate, systolic and diastolic blood pressure and ecocardiographic parameters between HD and PD patient groups (respectively table 1 and 2). Diabetes mellitus was found significantly more frequent (%32 versus 10% ; p= 0.042) and hypertension was tend to be more frequent (%60 vs %36.7 ;p=0.084) in HD patients. In PD patients dyslipidemia was significantly more frequent (40% versus 16% versus; p=0.047) while total cholesterol (203.96 ± 59.33 versus 162.00 ± 43.79;p=0.005), low-density lipoprotein (LDL) (118.36 ± 38.97 versus 95.44 ± 31.11; p=0.021) and triglyceride (258.73 ± 200.80 versus 174.20 ± 76.02 ;p=0.050) levels were significantly higher (Table 3). Aortic strain (8.72 ± 4.17 versus 9.65 ± 5.54; p=0.478), dystensibility (0.39 ± 0.21 versus 0.45 ± 0.26; p=0.329) and PWPV (13.7 ± 0.41 versus 12.9±0.47; p=0.512) were not significantly different between HD and PD patients (Table 4).

*Table 1. Demographical specifications of hemodialysis and peritoneal dialysis patients*

| | PD ( n = 30 ) | HD ( n = 25 ) | P value | |
|---|---|---|---|---|
| Age | 46.36 ± 14.99 | 39.96 ± 16.88 | 0.142 | |
| Length(cm) | 158.30 ± 8.33 | 161.92 ± 10.59 | 0.162 | |
| Weight(kg) | 65.30 ± 16.15 | 63.56 ± 16.00 | 0.691 | |
| RRP | 49.60 ± 25.35 | 45.40 ± 28.76 | 0.567 | |
| Systolic BP | 119.16 ± 24.00 | 129.60 ± 20.66 | 0.093 | |
| Diastolic BP | 76.83 ± 14.17 | 78.80 ± 15.43 | 0.625 | |
| Pulse/min. | 81.90 ± 11.57 | 81.96 ± 10.58 | 0.984 | |
| DM | 3 (10% ) | 8 (32% ) | 0.042 | Q-square: 4.125 |
| Hypertension | 11 (36,7% ) | 15 (60% ) | 0.084 | Q-square: 2.979 |
| Hyperlipidemia | 12 (40% ) | 4 (16% ) | 0.047 | Q-square: 3.808 |
| Gender; Male | 6 (20%) | 11 (44% ) | | |

*RRP: Renal replacement period, BP: Blood pressure , DM: Diabetes mellitus*

*Table 2. Echocardiograph parameters of hemodialysis and peritoneal dialysis patients.*

| | PD ( n = 30 ) | HD ( n = 25 ) | P value | |
|---|---|---|---|---|
| AoS | 2.87 ± 0.41 | 3.02 ± 0.46 | 0.199 | |
| AoD | 2.653 ± 0.43 | 2.76 ± 0.45 | 0.341 | |
| LA | 3.30 ± 0.50 | 3.36 ± 0.56 | 0.692 | |
| İVS | 1.12 ± 0.16 | 1.21 ± 0.15 | 0.037 | |
| PW | 1.097 ± 0.16 | 1.17 ± 0.12 | 0.072 | |
| LVDD | 4.30 ± 0.45 | 4.58 ± 0.54 | 0.052 | |
| LVSD | 2.74 ± 0.41 | 2.96 ± 0.43 | 0.070 | |
| LVH | 13 (43,3%) | 14 (56%) | 0.349 | Q-square: 0.875 |

*AoS: systolic aortic diameter, AoD: diastolic aortic diameter, LA: left atrium, İVS: interventricular septum, PW: posterior wall, LVDD: left ventricular diastolic dysfunction, LVSD: left ventricular systolic dysfunction, LVH: left ventricular hypertrophy*

*Table 3. Lab values of hemodialysis and peritoneal dialysis patients*

| | PD ( n = 30 ) | HD ( n = 25 ) | P value |
|---|---|---|---|
| Glucose | 103.80 ± 30.62 | 118.88 ± 36.52 | 0.102 |
| Cholesterol | 203.96 ± 59.33 | 162.00 ± 43.79 | 0.005 |
| LDL | 118.36 ± 38.97 | 95.44 ± 31.11 | 0.021 |
| HDL | 40.26 ± 10.51 | 37.34 ± 14.00 | 0.381 |
| Triglyceride | 258.73 ± 200.80 | 174.20 ± 76.02 | 0.050 |
| Na | 138.26 ± 2.84 | 138.20 ± 4.17 | 0.944 |
| K | 4.02 ± 0.62 | 4.23 ± 0.72 | 0.264 |

*LDL: low-density lipoprotein, HDL: high-density lipoprotein, Na: sodium, K: Potassium,*

*Table 4. Arterial stiffness parameters of hemodialysis and peritoneal dialysis patients*

| | PD ( n = 30 ) | HD ( n = 25 ) | P value |
|---|---|---|---|
| Aortic strain ( % ) | 8.72 ± 4.17 | 9.65 ± 5.54 | 0.478 |
| Dystensibility ($cm^2/dyn/10^3$) | 0.45 ± 0.26 | 0.39 ± 0.21 | 0.329 |
| Aorto-femoral PWPV (m/s) | 13.7 ± 0.41 | 12.9 ± 0.47 | 0.512 |

*PWPV: pulse-wave propagation velocity, PD: peritoneal dialysis, HD: hemodialysis*

**Discussion**

It is well known that ischemic cardiac disease, left ventricular hypertrophy, congestive cardiac failure, stroke and macro-vascular disorders responsible from the high incidence of mortality can develop faster in patients with uremia [15]. In a study carried out by Locatelli et al [3], it was concluded that cardiovascular diseases mentioned above which are considered as the most significant reason of morbidity and mortality in ESRD were related with atherosclerosis. Early diagnosis of atherosclerosis can be realized by non-invasive methods of mechanical specifications of the aorta. Nicole et al [16] revealed that aorta stiffness can be used as an indicator of atherosclerosis.

Measurements of arterial stiffness may play a major role in the classification of a better risk assessment because of a risk due to a cardiovascular disease and in initiating early preventive therapy [17]. As an early preventive therapy, we recommend to use angiotensin-converting enzyme inhibitor and statin drugs which are recognized, firstly for the elastic characteristics of their arterial wall and their well-known definite beneficiary effects on the endothelial function [18]. Arterial stiffness demonstrates an increase by age [19] and by an intravascular tension pressure. Similarly, aortic elasticity may become influenced by the course of certain disorders such as, ESRD [20], hypertension [21], ateroskleroz [22] and diabetes mellitus [23]. In ESRD patients, arterial stiffness is related with an increase in the wall thickness of major arteries and increased arterial aging accompanied with dilatation [17].

Arnett et al [4] demonstrated that arterial stiffness which is recognized as an indicator of atherosclerosis is an important risk factor in the development of cardiovascular morbidity and mortality. In a study carried out by Stefanadis et al [13], pressure-diameter relationships and aortal elasticity were assessed by the aid of invasive and non-invasive methods and aortic elasticity was depicted as a strong and independent risk factor in repeated coronary events in patients with coronary artery diseases. Similarly, Jacques Blacher et al [17] showed that, arterial stiffness was a major predictive factor in mortalities due to cardiovascular and entire reasons in hemodialysis patients. Nevertheless, in the current study arterial stiffness was assessed with the pulse-wave propagation velocity was displayed as a better predictor factor for mortality when compared to other factors such as age and hemodialysis period.

Arterial stiffness and changes in dystensibility can be evaluated by invasive methods or by certain formulas based on the relationship of pressure and diameter . Aortic dystensibility and aortic strain which can be calculated by echocardiography is a non-invasive method used to determine arterial elasticity [24]. The measurement of the pulse-wave propagation velocity is also another non-invasive method used in previous studies related with arterial stiffness [25,26]. In the mentioned studies, aortic pulse-wave propagation velocity was demonstrated as a survival indicator in HD patients [27,28] and in patients with essential hypertension [29,30].

In a study carried out by Adrian Covic et al [7] on 41 HD and 41 PD patients, the level of arterial stiffness was found higher in PD patients when compared against HD patients and the difference in arterial elasticity was assumed to be related with the difference of the renal replacement modality applied. Still, in some other studies [8,9] similar results were obtained which left ventricular hypertrophy associated with increased arterial stiffness was higher in PD patients. The mentioned condition was considered to be related with the excess volume accumulation in PD patients [8,31]. The status of volume, blood pressure and arterial stiffness/dystensibility are three major indicators of after-load and pre-load. In normal individuals or in

dialysis patients arterial stiffness and endothelium dependent vasorelaxation impairment or continuous volume excess may lead to an increase in left ventricular mass [32,33]. Yet, in our study we determined no any difference between PD and HD patient groups regarding left ventricular hypertrophy.

Konings et al [10] compared 18 HD, 36 PD and 25 control patients in a study and determined that the dystensibility of the right common carotid artery was similar to the dystensibility in the PD and control group of patients, but that it was significantly lower in hemodialysis patients. It was stated that this difference could be dependent to a higher levels of DM, cigarette smoking and increased prevalence of macro vascular diseases among the patient population who treatment with HD patients and the different technique used to make measurements from the common carotid artery, instead from the aorta.

In a study carried out by Wilkinson et al [34], dyslipidemia was found related with increased aortic stiffness. In another study performed by Adrian Covic et al [7], similar to the findings in our study, cholesterol levels were found significantly higher in PD patients when compared to HD patients and parallel to this finding, aortic stiffness was more intense in PD patients.

In our study, hypertension frequency somehow showed a higher propensity in HD patients while the ratio of patients diagnosed with DM was significantly higher when compared to patients with PD.

The ratio of patients diagnosed with dyslipidemia was higher in PD patients while it was determined that cholesterol, LDL and triglyceride levels were higher in PD patients when compared to HD patients. However, together with this findings and opposite with the findings found in recent studies, we failed to determine a significant difference in aortic stiffness parameters such as aortic strain, dystensibility and pulse-wave propagation velocity among two patient groups.

Consequently, we assumed that there was no any significant difference between HD and PD therapeutical modalities regarding their effect on arterial stiffness, whereas recent studies made us think that factors effecting arterial stiffness may originate from the differentiability of certain factors among patient groups, such as age, hypertension, hyperlipidemia and diabetes mellitus.

## References


1. *United States Renal Data System. USRDS Annual data Report. 2005.*
2. *Zoccali C. Cardiovascular risk in uraemic patients is it fully explained by classical risk factors? Nephrol Dial Transplant 2000; 15: 454-7.*
3. *Locatelli F, Marcelli D, Conte F et al. Cardiovascular disease in chronic renal failure: the challenge continues. Nephrol Dial Transplant 2000; 15: 69-80.*
4. *Arnett DK, Evans GW, Rıley WA. Arterial stiffness a new cardiovascular risk factor. Am J Epidemiol. 1994; 40 (8): 669-82.*
5. *Belz GG. Elastic properties and Windkessel function of the human aorta. Cardiovasc. Drugs Ther. 1995; 9: 73-83.*
6. *Breıthaupt-Grögler K. , Belz G.G. Epidemiology of the arterıal stıffness. Pathologıe Biologie. 1999; 47: 604-13.*
7. *Adrian Covic, David J.A. Goldsmith, Laura Florea, Paul Gusbeth–Tatomir, and Maria Covic The Influence of Dialytic Modality on Arterial Stiffness, Pulse Wave Reflections, and Vasomotor Function. Perit Dial Int 2004; 24: 365–72*
8. *Enia G, Mallamaci F, Benedetto FA, Panuccio V, Parlongo S, Cutrupi S, et al. Long-term CAPD patients are volume-expanded and display more severe left ventricular hypertrophy than hemodialysis patients. Nephrol Dial Transplant 2001; 16: 1459–64.*
9. *Takeda K, Nakamoto M, Hirakata H, Baba M, Kubo M, Fujishima M. Disadvantage of long-term CAPD for preserving cardiac performance: an echocardiographic study. Am J Kidney Dis 1998; 32: 482–7.*
10. *Konings CJ, Dammers R, Rensma PL, Kooman JP, Hoeks AP, Kornet L, et al. Arterial wall properties in patients with renal failure. Am J Kidney Dis 2002; 39: 1206–12.*
11. *Lindner A, Charra B, Sherrard DJ, Scribner BH. Accelerated atherosclerosis in prolonged maintenance hemodialysis. N Engl J Med. 1974; 290: 697–701.*
12. *Nicole M, Van Popole MD, Diederick E. Association between arterial stiffness and atherosclerosis. The Rotterdam Study Stroke. 2001; 32: 454-60 .*
13. *Jacques Blacher, MD; Alain P. Guerin, MD; Bruno Pannier, MD; Sylvain J. Marchais, MD; Michel E. Safar, MD; Ge´rard M. London, MD Impact of Aortic Stiffness on Survival in End-Stage Renal Disease. Circulation. 1999; 99: 2434-39.*

14. *London GM, Pannier BM, Guerin AP, Marchais SJ, Safar ME, Cuche J-L. Cardiac hypertrophy, aortic compliance, peripheral resistance, and wave reflection in end-stage renal disease — comparative effects of ACE inhibition and calcium channel blockade. Circulation 1994; 90: 2786–96.*

15. *Nichols WW, O'Rourke MF. Vascular impedance. In: McDonald's Blood Flow in Arteries: Theoretical, Experimental and Clinical Principles. 4th ed. London, UK: Edward Arnold; 1998: 243–83.*

16. *London GM, Gue´rin AP, Marchais SJ, Pannier B, Safar ME, Day M, Metivier F. Cardiac and arterial interactions in end-stage renal disease. Kidney Int. 1996; 50: 600–608.*

17. *Safar ME, Frohlich ED. The arterial system in hypertension: a prospective view. Hypertension. 1995; 26: 10-14.*

18. *Wada T, Kodaira K, Fujishiro K, Maie K, Tsukiyama E, Fukumoto T, Uchida T, Yamazaki S. Correlation of ultrasound-measured common carotid artery stiffness with pathological findings. Arterioscler Thromb. 1994;14: 479–82.*

19. *Mulvany M.J A reduced elastic modulus of vascular wall components in hypertension? Hypertension, 1992; 20: 7-9.*

20. *Lacombe F, Dart A, Dewar E, Jennings G. Cameron J, et al. Arterial elastic properties im amn: a comparison of echo-Doppler indices of aortic stiffness. Eur Heart J 1992; 13: 1040-45.*

21. *Blacher J, Asmar R, Djane S, et al. Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. Hypertension 1999; 33: 1111-7.*

22. *Asmar R, Benetos A, Topouchian J, Laurent P, Pannier B, Brisac A-M, Target R, Levy BI. Assessment of arterial distensibility by automatic pulse wave velocity measurement: validation and clinical application study. Hypertension. 1995; 26: 485– 90*

23. *Blacher J, Guerin AP, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness on survival in end-stage renal disease. Circulation 1999; 99: 2434–9.*

24. *London GM, Blacher J, Pannier B, Guerin AP, Marchais SJ, Safar ME. Arterial wave reflections and survival in end-stage renal failure. Hypertension 2001; 38: 434–8.*

25. *Boutouyrie P, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P, et al. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. Hypertension 2002; 39: 10-15.*

26. *Laurent S, Boutouyrie P, Asmar A, Gautier I, Laloux B, Guize L, et al. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension 2001; 37: 1236–41.*

27. *Sahn DJ, DeMaria A, Kisslo J, Weyman A. The committee on M-mode standardization of the American Society of Echocardiography: recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographicmeasurements. Circulation, 1978; 58: 1072-83.*

28. *Lacombe F, Dort A, Dewar E, et al. Arterial elastic properties in man: a comparison of echo-Doppler indices of aortic stiffness. Eur 1992; 13: 1040-5.*

29. *Stefanadis C, Wooley CF, Dush CA, et al. Aortic distensibility in post stenotic aortic dilatation: J. Cardiol 1988; 18: 78-82.*

30. *Konings CJ, Kooman JP, Schonck M, Dammers R, Cheriex E, Palmans Meulemans AP, et al. Fluid status, blood pressure, and cardiovascular abnormalities in patients on peritoneal dialysis. Perit Dial Int 2002; 22: 477–87.*

31. *Covic A, Goldsmith DJ, Panaghiu L, Covic M, Sedor J. Analysis of the effect of hemodialysis on peripheral and central arterial pressure waveforms. Kidney Int 2000; 57: 2634–43.*

32. *London GM. Left ventricular alterations and end-stage renal disease. Nephrol Dial Transplant 2002; 17: 29–36.*

33. *Lehmann ED, Hopkins KD, Gosling RG: Aortic compliance measurements using Doppler ultrasound: In vivo biochemical correlates. Ultrasound Med Biol, 1993; 19: 683–710*

34. *Wilkinson IB, Hall IR, MacCallum H, Mackenzie IS, McEniery CM, van der Arend BJ, et al. Pulse-wave analysis: clinical evaluation of a noninvasive, widely applicable method for assessing endothelial function. Arterioscler Thromb Vasc Biol 2002; 22: 147–52.*

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# Scutellarein induces apoptosis of SAS human tongue cancer cells via activating mitochondrial signaling pathway

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**Abstract**

To investigate the mechanism in Scutellarein-induced apoptosis of SAS human tongue cancer cells，inhibitory effects of Scutellarein on SAS cells were detected by MTT assay. Cell apoptosis was analyzed by flow cytometry. Ultrastructural changes of SAS cells were observed by transmission electron microscopy (TEM). Mitochondrial transmembrane potential (△Ψm) were analyzed by JC-1 [5,5,6,6-Tetrachloro-1,1,3,3-tetraethylbenzimidazolylcarbocyanine iodide]. Western blotting was used to examine the expression level of Bcl-2, Bax and caspase-3 in SAS cells treated with Scutellarein. Scutellarein inhibited the proliferation of SAS cells in a time and dose-dependent manner and increased the percent of apoptotic cells. The mitochondrial cristae were swollen and had vacuolar degeneration. □Ψm was decreasing when the concentration of scutellarein was increasing. Scutellarein effectively up-regulated the expression of mitochondrial Bax and caspase-3 and down-regulated the expression of Bcl-2. Scutellarein induces apoptosis of SAS human tongue cancer cells via activating mitochondrial signaling pathway.

**Key words:** Scutellarein, mitochondria, apoptosis, SAS Tongue cancer cell.

## Introduction

Oral squamous cell carcinoma (OSCC) is the most common head and neck cancer and known to have poor prognosis which caused by local invasion and distant metastasis[1]. Although extensive research is being done on its pathogenesis and management, the 5-year survival rate for OSCC patients only improved to 53% over the past decades[2]. Chemotherapy is a standard and adjunctive treatment for surgical treatment when tumor metastasis has involved the lymph nodes or distant organs, but the treatment has toxic side effects on many patients[3]. Numerous cancer research studies have focused on traditional medicinal plants in an effort to discover new therapeutic agents that lack the toxic side effects compared with current chemotherapeutic agents.

Flavonoids, a large group of aromatic plant secondary metabolites that produced from traditional Chinese medicinal plants, have shown the compelling data for the antitumor activities[4, 5]. Extracts and isolated flavonoids from herb could relieve oxidative stress and immune dysfunction associated with the onset and progress of cancer[6]. Studies also demonstrated that flavonoids were able to arrest the cell cycle of tumor cell lines that are resistant to multiple chemotherapeutic drugs and inhibit the progression of tumor angiogenesis[7, 8].

The most versatile plants used as a source of flavonoids are *S. baicalensis* and *S. barbata*, the members of genus Scutellaria family. It has been reported that *S. baicalensis* and *S. barbata* have diverse biological activities including anti-inflammatory[9], antioxidative[9], and antibacterial[10]. Recent investigations show that *S. baicalensis* and *S. barbata* alone, or in combination with other herbs, can inhibit cancer cell proliferation or induce apoptosis in breast[11], hepatocellular[12], lung[13], prostate[14], colon[15], and gynecologic neoplasms[16]. But, it is still unclear about whether these activities are caused by the additive or synergistic effects of several components of *S. baicalensis* or *S. barbata*, or due to a single component of them.

Flavonoids, containing baicalein, baicalin, apigenin, scutellarein, scutellarin, wogonin, luteolin and apigenin, *et al*, constitute the major phyto-

chemical component of Scutellaria extracts. Preclinical studies have demonstrated that baicalein has a favorable effect in cisplatin-induced cell death of human glioma cells[17]. It also can induce apoptosis of myeloma cell lines via mitochondria pathway by inhibiting the phosphorylation of IkB-alpha [18]. Apigenin can inhibit growth of pancreatic cancer cells through suppression of cyclin B-associated cdc2 activity and G2/M arrest[19]and induce breast cancer cells apoptosis through proteasomal degradation of HER2/neu[20]. Apigenin and luteolin induce apoptosis by down-regulating the Pharmacologica Sinica IGF-I expression, which contributes to the selective growth of leiomyoma[21]. Luteolin induces apoptosis of Lewis lung carcinoma cells by effectively activating caspase 9 and 3 and down-regulating the expression of extracellular signal-regulated kinase (ERK) and Akt[22]. Wogonin can inhibit phorbol 12-myristate 13-acetate-induced COX-2 gene expression in human lung epithelial cancer A549 cells[13] and promote the granulocytic differentiation of human promyelocytic leukemia cells by up-regulating the expression of phospholipid scramblase 1 gene[23]. Scutellarein, 5,6,7,4'-Tetrahydroxy flavone, is a flavonoid monomer composition of *S. baicalensis* and *S. barbata*. The antitumor properties of it have rarely been studied. To explore the effects of scutellarein on the human tongue cancer SAS cells, we examined the effects of it on cells proliferation and apoptosis in vitro, and the morphology and function changes of mitochondria, and apoptosis-related protein expression of bcl-2, bax, and caspase 3 on mitochondrial pathways.

## Materials and methods

### *Materials*

Scutellarein (purity 99%, HPLC) was purchased from Guangzhou BiomolBio-Tec Co. Ltd. (Guangzhou, China). SAS human tongue cancer cell lines were from the Human Science Research Resources Bank (Osaka, Japan). RPMI-1640 and FBS were from Gibco BRL (Life Technologies, Paisley, Scotland). Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich (St. Louis, MO). MTT was from KeyGen (Nanjing, China). Annexin V-FITC apoptosis detection kit was from BD Biosciences (San Diego, CA). Antibodies against bcl-2, bax, caspase 3, and actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Secondary antibodies were purchased from Sigma (St. Louis, MO). Enhanced chemiluminesence (ECL) reagent was purchased from Amersham International (Amersham, UK). All other reagents and solvents used in experiments were of analytical grade.

### *Cell culture*

Cells were maintained at 37 °C in a humidified incubator (Heraeus, Germany) containing 5% CO2, in Roswell Park Memorial Institute 1640 (RPMI-1640) medium, supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin.

### *MTT assay*

Cells were plated at a density of 10,000 cells in 96-well plates. After incubation for 24 h, the cells were treated with different concentrations (5, 25, 125 and 625 μM) of scutellarein for 24, 48 and 72 h. Drug cytotoxicity was evaluated by using a MTT assay. Fifty μl MTT was added to each well at 5 mg/ml, incubation was continued for 4 h. Formazan crystals resulting frommitochondrial enzymatic activity on MTT substrate were solubilized with 150 μl DMSO and absorbance was measured at 490 nm using Stat Fax2100 microplate reader (Equl-Awaretech, USA). Each drug concentration was set upin six replicate wells, and experiments were repeated three times. Cell survival was expressed as absorbance relative to that of untreated controls.

### *Flow Cytometric Analysis*

After treatment with 25 and 125 μM of scutellarein for 48 h, cells were collected and washed with cold PBS. The cells were labeled by incubation with 5 mL FITC-annexin V and 10 mL PI at 250 mg/mL for 10 min in the dark at room temperature. The cells were washed with PBS again and examined by flow cytometry (BD Biosciences). Apoptosis was routinely quantified by counting the number of cells stained with FITC-labeled annexin V.

### *Transmission electron microscopy*

Treated with 25 and 125 μM of scutellarein for 48 h, the cells were fixed in 40% glutaraldehyde solution and postfixed in 1% osmium for 2 h be-

fore they were immersed with Epon 821 for 72 h at 60 ℃. After that, the cells were sectioned into ultrathin slices (60 nm) and stained with uranyl acetate and lead citrate. Cell morphology was observed using TEM.

Mitochondrial depolarization assay

The cells treated with scutellarein (25 and 125 μM) for 48 h were incubated with an equal volume of JC-1 [5,5,6,6-Tetrachloro-1,1,3,3-tetraethylbenzimidazolylcarbocyanine iodide] staining solution (5 mg/ml) at 37 °C for 20 min and rinsed twice with PBS. Mitochondrial membrane potentials were monitored by determining the relative amounts or dual emissions from both mitochondrial JC-1 monomers and aggregates under an Olympus fluorescent microscope at 488 nm excitation. Mitochondrial depolarization was indicated by an increase in the green/red fluorescence intensity ratio.

*Western blot analysis*

After treated with different concentrations (25 and 125 μM) of scutellarein for 48h, cells were lysed by 100 μl lysis buffer (Tris 50 mM, pH 7.4, NaCl 150 mM, TritonX-100 1%, EDTA 1 mM, and PMSF 2 mM) and incubated for 30 min on ice. The lysates were sonicated and centrifuged at 16,099g for 10 min, and the supernatants were collected and stored at –80 °C before they were used in Western blot analysis. The protein concentrations were determined by the Bradford assay using bovine serum albumin as standard. Cells protein samples containing 20 μg of protein were separated by SDS-PAGE and electro blotted ontonitrocellulose sheets, blocked for 1 h at room temperature with a buffer (20 mM Tris , 150 mM NaCl, pH 7.6, 0.1% Tween 20) containing 5% nonfat dry milk and then probed with appropriate antibodies to bcl-2, bax, procaspase 3 andactin at 1:500 dilution overnight at 4 °C followed by horseradishperoxidase-conjugated goat anti-rabbit IgG (1:50,00 dilution) or goat anti-mouse IgG (1:50,00 dilution) antibodies for 1 h at room temperature. Immunoreactivity was detected using ECL Western blotting detection kit and exposed to X-ray film. Immunoblots were scanned using a GS-800 densitometer and protein bands were quantified with Quantity One software (Bio-Rad Laboratories, Hercules, CA).

***Statistical analysis***

Experiments were independently performed at least 3 times. Data are presented as the mean ± SEM. Data were analyzed using one way ANOVA. Statistical significance was established at $P<0.05$.

## Results

***Scutellarein induce apoptosis of SAS cells***

MTT assay was used to examine the effect of scutellarein on cell viability. As shown in Fig 1, scutellarein inhibited SAS cell viability significantly in a dose- and time-dependent manner when compared with the DMSO-treated control. The difference was significant for cells treated with 25 and 125 μM of scutellarein and incubated for 48 h, compared with the control group ($P<0.05$). Flow Cytometric analysis showed that the percentages of early and late apoptotic cells was significantly increased after treated with 25 and 125 μM scutellarein for 48 h (Figure 2).

***Effect of Scutellarein on cell morphology***

To confirm the morphological changes of apoptosis, Ultrastructural changes of SAS cells treated with various Scutellarein for 48 h were observed by TEM. The control cells have large nucleus, rich chromatin and microvilli, and integral organelles (Figure 3a). Treated with 25 μM Scutellarein, the cells showed early morphological changes in apoptosis including nucleus shrunk, chromatin condensation, mitochondrial swelled and mitochondrial myeloid degeneration (Figure 3b and 3c). Microvilli loss, nuclear chromatin crescent-like clumps, vacuolar degeneration, nuclear membrane splitting, nuclei fragmentation, and apoptotic bodies were observed when cells were treated with 125 μM Scutellarein (Figure 3d and 3e).

***Scutellarein promoted mitochondrial depolarization***

Mitochondrial function was assessed with the mitochondrial $\triangle\Psi m$ measured with JC-1 red fluorescence. Relative mitochondrial mass was determined by a fluorescent microscope using JC-1. The cells were stained with JC-1 probe for the measurement of mitochondrial depolarization. The quantitative analysis of JC-1-stained cells revealed a significant decrease in the red (high $\triangle\Psi m$) to

green (low △Ψm) ratio in inscutellarein-treated cells when compared with control cells (Figure 4A). The expression of Bcl-2 and Bax was associated with mitochondrial function. Western blotting assay showed that Bcl-2 expression in SAS cells was significantly suppressed after treatment for 48 h with 25 and 125 μM Scutellarein, showing a negative correlation with the Scutellarein dosage (Figure 5A, 5B, 5D, n=3, $p<0.05$), while Bax expression was significantly increased, showing a positive correlation with the Scutellarein dosage (Figure 5, n=3, $p<0.05$). The expression of caspase-3 was assayed for the purpose of confirming the apoptosis. As shown in Figure 5, after treatment with Scutellarein for 48 h, the expression of caspase-3 remarkably increased in a dose-dependent manner detected by western blot assay (Figure 5, n=3, $p<0.05$). These results indicated that Scutellarein did induce apoptosis in a population of SAS cells.

## Discussion

Apoptosis is required for correct development and organismal homeostasis, while malfunction of apoptosis contribute to the progression of cancer and degenerative diseases [24]. Because many chemotherapeutic drugs could induce apoptosis in malignant cells, apoptosis has currently been a target for developing antitumor drugs [25]. Recently, there is a growing interest in the use of natural materials or naturally occurring substances for the treatment or prevention of cancer because of the few side effects. Study shows that Scutellarein can inhibit human malignant glioma cell lineU87-MG and breast carcinoma MDA-MB-231 proliferation, while without affecting human mammary epithelial cell and prostate cancer cell line PC3[26]. Therefore, we suspected that the antitumor activity and mechanism of Scutellarein on tongue cancer might not be the same with other tumor cells.

In this study, we used MTT to verify the effect of scutellarein on SAS cell proliferation. Scutellarein was found to inhibit SAS cell viability significantly in a dose- and time-dependent manner (Figure 1) and Flow cytometric analysis indicated that the quantity of apoptotic cells increased in according to the increasing concentrations of scutellarein (Figure 2). Apoptotic cells are characterized by cell shrinkage, membrane blebbing and chromatin condensation culminating in cell fragmentation [27], which are the distinctive morphological features to confirm the apoptosis and its extent. SAS cells also exhibited morphological changes from early to late apoptosis when treated with different concentration scutellarein detected by TEM (Figure 3b-3e). These results indicate that the proliferation inhibition effect of scutellarein on SAS cells was due to the induction of apoptosis.

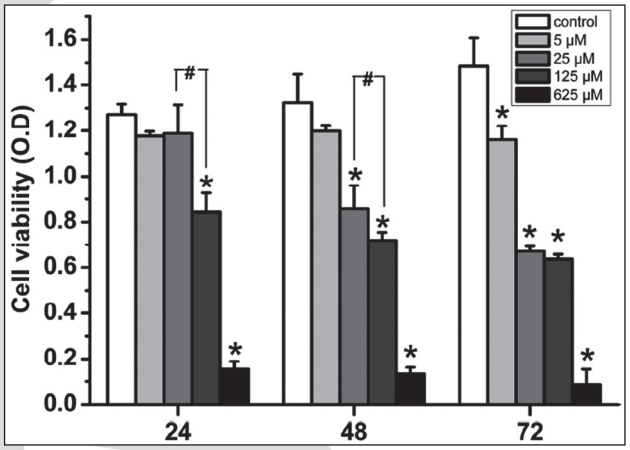


*Figure 1. Scutellarein cytotoxicity on SAS cells. Cells were treated with various concentrations of scutellarein (5, 25, 125, 625 μM) and incubated for 24, 48 and 72 h. Scutellarin cytotoxicity was detected using MTT degradation assay, shown by absorbance at 490 nm. Data represent the mean ±SEM of three independent experiments. *P<0.05 versus control, #P<0.05 versus each other.*

The ultra-morphological changes were that the mitochondrial swelling gradually increased and mitochondrial crest was dissolved to promote myeloid changes and vacuolar degeneration when the concentration of scutellarein was increased from 25 and 125 μM (Figure 3b-3d). Mitochondria are important intracellular organelles to supply energy and regarded as central regulator of the decision between cellular survival and demise[28]. Mitochondrial structure changed significantly when cell apoptosis occurred. Mitochondrial cristae may lose packed and ordered folding. The membrane gap is widened and filled with concentrated Matrix. Mitochondria appear extensive swelling and have vacuolar degeneration[29].

Mitochondrial dysfunction is characterized by the loss of △Ψm and that is an early event in the cell apoptotic process.△Ψm was decreased before

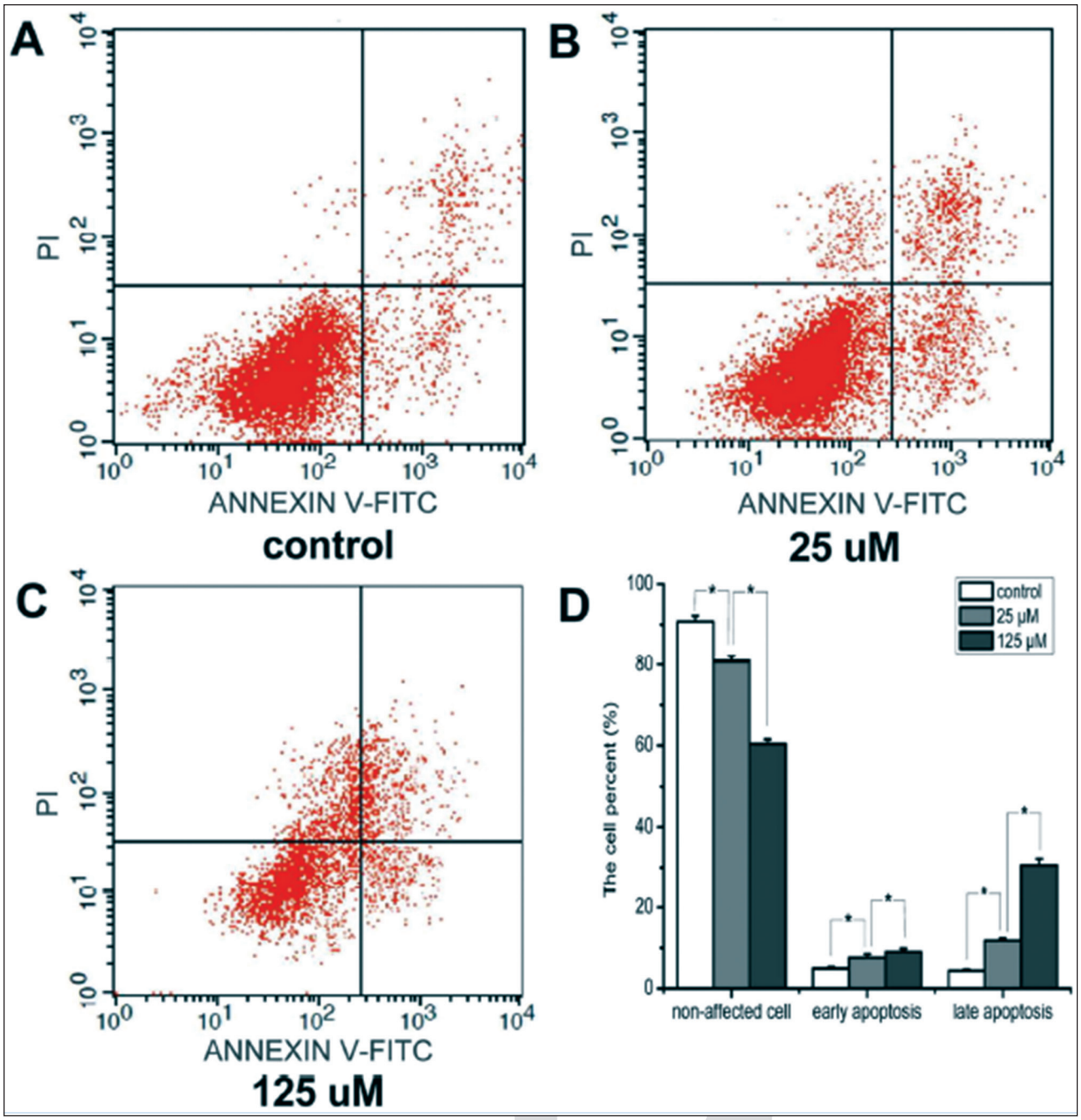


*Figure 2. Effect of Scutellarein on the apoptosis of SAS cells. Apoptosis of SAS cells shown by annexin-V/PI staining after treated with 25 and 125 μM scutellarein for 48 h, with data showing the percentages of non-affected, early and late apoptotic cells. All values are given as mean ± SEM from three independent experiments. *P<0.05 compared with each other.*

chromatin condensation and DNA fragmentation. Reduced △Ψm induces Cyt-c release from the mitochondria, and causes apoptosis[30]. In this study, scutellarein significantly decreased the △Ψm (Figure 4), suggesting that scutellarein induced apoptosis of SAS cells via the mitochondrial pathway. Loss of △Ψm was found to be closely associated with the expression of Bcl-2and Bax. The Bcl-2 family protein, localized to the outer mitochondrial membranes, controls the stabilization of mitochondrial membranes (Figure 5). The expression of Bax was positive correlated with decreased ΔΨm and the expression of Bcl-2 was negatively correlated with decreased ΔΨm, suggesting that increased Bax expression or decreased Bcl-2 expression may be involved in the decreased ΔΨm. Increased Bax/Bcl-2 proportion will lead to Cyt-c release from the mitochondria, thus inducing cell

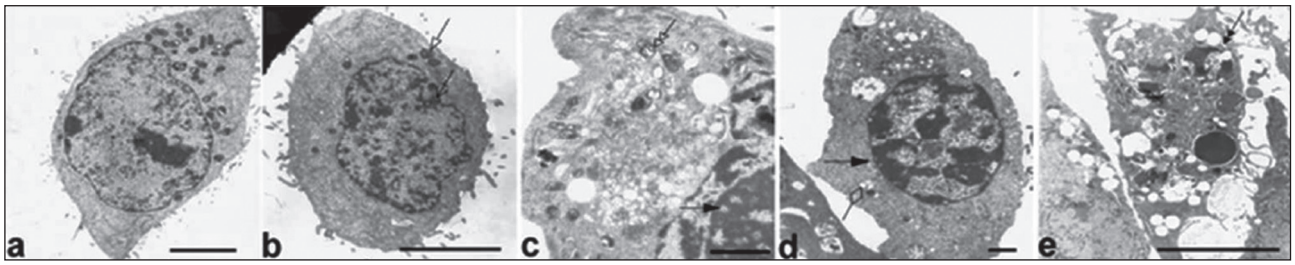


*Figure 3. Effects of scutellarein on the Ultrastructural changes of SAS cells. Cells were treated with various concentrations of scutellarein (25, 125μM) and incubated for 48 h. The morphologic change of SAS Cells was detected by transmission electron microscopy. a, Control cells have large nucleus, rich chromatin and microvilli, and integral organelles. b, c: Nucleus shrunk (↓), chromatin condensation (↓), mitochondria swelled (↓) and even mitochondrial crest dissolved to form myeloid changes (↓) after treatment with 25 μM scutellarein. d, e: Microvilli defluxion, chromatin margination plaque, mitochondrial vacuolar degeneration (↓) and even apoptotic body (↓) formed after treatment with 125 μM scutellarein. a, b and e: Bar=5 μm; c and d: Bar=1 μm*

apoptosis[31]. *S. barbata* might induce tumor cells apoptosis by suppressing the expression of bcl-2 or increasing the ratio of bax/bcl-2[32].

Extrinsic pathway and intrinsic pathway are two major pathways leading to cell apoptosis. Caspase family is central to the proteolytic events of apoptosis and it can be activated by an apoptosis death stimulus. Activation of initiator of caspases (procaspases 8-10) led to proteolytic activation of downstream effector caspases (caspase-3, -6, -7). Especially, the activation of caspase-3 is a common event in both pathways. As detected in many apoptotic tumor cells induced by *S. barbata*[33], our studies also showed that caspase 3 was activated (Figure 5). It indicated that exogenous scutellarein induces apoptosis of SAS cells.

In summary, scutellarein can up-regulate the expression of Bax and down-regulate the expression of Bcl 2, promote mitochondrial depolarization and damage mitochondrial structure, induce SAS cells apoptosis. It implied that scutellarein can induce apoptosis of tongue cancer SAS cells in vitro through activating mitochondria pathway. Its effectiveness demonstrated here suggests that scutellarein may be a promising chemotherapy agent in inhibiting tumor growth.

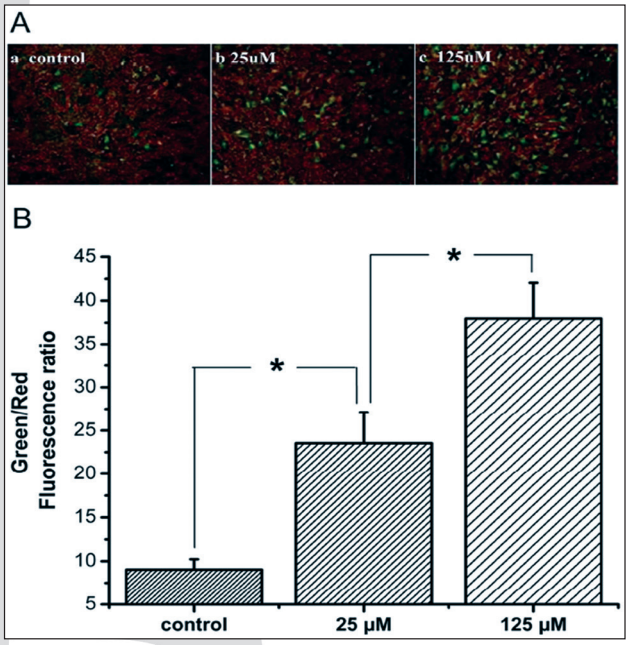


*Figure 4. Effects of scutellarein on the ΔΨm of human tongue cancer SAS cells. SAS cells were treated with scutellarein (5, 25 μM) for 48 h and stained with JC-1 probe and imaged by a fluorescent microscope. The individual red and green average fluorescence intensities are expressed as the ratio of green to red fluorescence. The increase of fluorescence ratio, which is represented in the bars, is correlating with an increase in mitochondrial depolarization a-c. representative photographs of JC-1 staining in different groups. Quantitative analysis of the shift of mitochondrial green fluorescence to red fluorescence among groups. All values are denoted as mean ±SEM from ten independent photographs shot in each group. *P<0.05 compared with each other.*

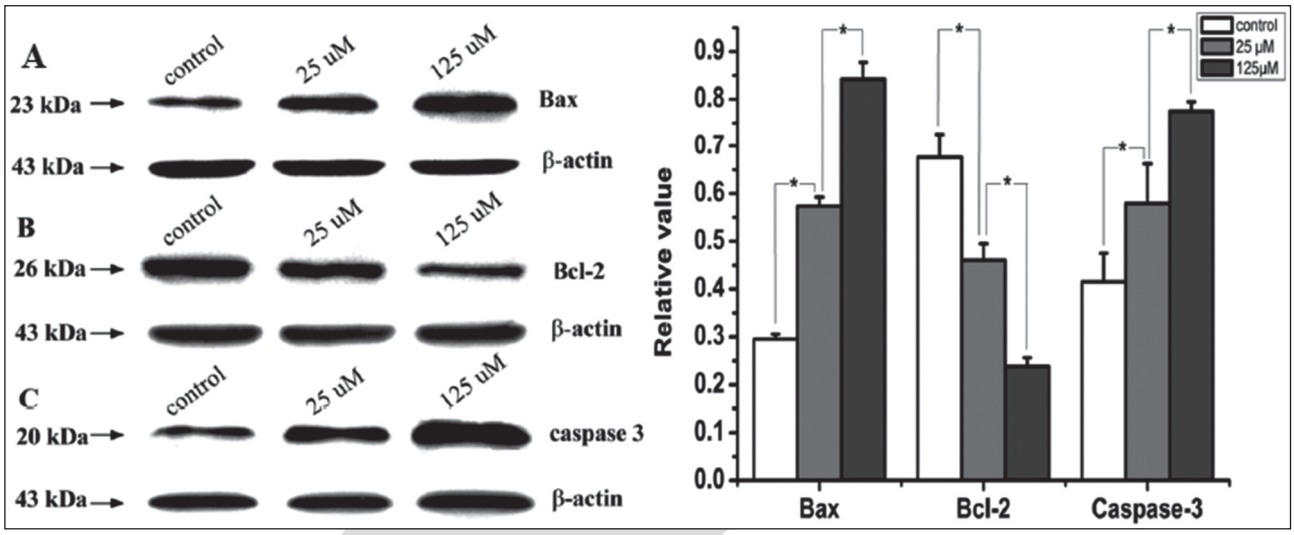


*Figure 5. Effects of scutellarein on protein expression levels of Bax, Bcl-2 and Caspase-3 in SAS cells. SAS cells were treated with scutellarein (5, 25μM) for 48 h. The protein expression levels of Bax, Bcl-2 and Caspase-3 were evaluated by western blotting assay. β-actin was used as an internal control. The expression levels of Bax and Caspase-3 increased as scutellarein concentration rose while the expression levels of Bcl-2 decreased compared to controls. Immunoblots were scanned using a GS-800 densitometer and protein bands from three independent experiments were quantified with Quantity One software (Bio-Rad Laboratories, Hercules, CA). All values were expressed as mean ± SEM. *P<0.05 compared with each other.*

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**References**

1. *Myoung H, Kim MJ, Hong SD, et al: Expression of membrane type I-matrix metalloproteinase in oral squamous cell carcinoma. Cancer Lett 2002; 185: 201-209.*

2. *Miyazaki Y, Hara A, Kato K, et al: The effect of hypoxic microenvironment on matrix metalloproteinase expression in xenografts of human oral squamous cell carcinoma. Int J Oncol 2008; 32: 145-151.*

3. *Panchal RG: Novel therapeutic strategies to selectively kill cancer cells. Biochem Pharmacol 1998; 55: 247-252.*

4. *Sun MY, Zuo J, Duan JF, et al: [Antitumor activities of kushen flavonoids in vivo and in vitro]. Zhong Xi Yi Jie He Xue Bao 2008; 6: 51-59.*

5. *Wang T, Zhang JC, Chen Y, et al: [Comparison of antioxidative and antitumor activities of six flavonoids from Epimedium koreanum]. Zhongguo Zhong Yao Za Zhi 2007; 32: 715-718.*

6. *Lahiri-Chatterjee M, Katiyar SK, Mohan RR, et al: A flavonoid antioxidant, silymarin, affords exceptionally high protection against tumor promotion in the SENCAR mouse skin tumorigenesis model. Cancer Res 1999; 59: 622-632.*

7. *Choi SU, Ryu SY, Yoon SK, et al: Effects of flavonoids on the growth and cell cycle of cancer cells. Anticancer Res 1999; 19: 5229-5233.*

8. *Liu JJ, Huang TS, Cheng WF, et al: Baicalein and baicalin are potent inhibitors of angiogenesis: Inhibition of endothelial cell proliferation, migration and differentiation. Int J Cancer 2003; 106: 559-565.*

9. *Huang WH, Lee AR, Yang CH: Antioxidative and anti-inflammatory activities of polyhydroxyflavonoids of Scutellaria baicalensis GEORGI. Biosci Biotechnol Biochem 2006; 70: 2371-2380.*

10. *Yu J, Lei J, Yu H, et al: Chemical composition and antimicrobial activity of the essential oil of Scutellaria barbata. Phytochemistry 2004; 65: 881-884.*

11. *Wang CZ, Li XL, Wang QF, et al: Selective fraction of Scutellaria baicalensis and its chemopreventive effects on MCF-7 human breast cancer cells. Phytomedicine 2010; 17: 63-68.*

12. *Ye F, Che Y, McMillen E, et al: The effect of Scutellaria baicalensis on the signaling network in hepatocellular carcinoma cells. Nutr Cancer 2009; 61: 530-537.*

13. *Chen LG, Hung LY, Tsai KW, et al: Wogonin, a bioactive flavonoid in herbal tea, inhibits inflammatory cyclooxygenase-2 gene expression in human lung epithelial cancer cells. Mol Nutr Food Res 2008; 52: 1349-1357.*

14. *Liu XH, Kirschenbaum A, Yao S, et al: Inhibition of cyclooxygenase-2 suppresses angiogenesis and the growth of prostate cancer in vivo. J Urol 2000; 164: 820-825.*

15. *Goh D, Lee YH, Ong ES: Inhibitory effects of a chemically standardized extract from Scutellaria barbata in human colon cancer cell lines, LoVo. J Agric Food Chem 2005; 53: 8197-8204.*

16. *Lee TK, Kim DI, Song YL, et al: Differential inhibition of Scutellaria barbata D. Don (Lamiaceae) on HCG-promoted proliferation of cultured uterine leiomyomal and myometrial smooth muscle cells. Immunopharmacol Immunotoxicol 2004; 26: 329-342.*

17. *Lee SW, Song GS, Kwon CH, et al: Beneficial effect of flavonoid baicalein in cisplatin-induced cell death of human glioma cells. Neurosci Lett 2005; 382: 71-75.*

18. *Ma Z, Otsuyama K, Liu S, et al: Baicalein, a component of Scutellaria radix from Huang-Lian-Jie-Du-Tang (HLJDT), leads to suppression of proliferation and induction of apoptosis in human myeloma cells. Blood 2005; 105: 3312-3318.*

19. *Ujiki MB, Ding XZ, Salabat MR, et al: Apigenin inhibits pancreatic cancer cell proliferation through G2/M cell cycle arrest. Mol Cancer 2006; 5: 76.*

20. *Way TD, Kao MC, Lin JK: Apigenin induces apoptosis through proteasomal degradation of HER2/neu in HER2/neu-overexpressing breast cancer cells via the phosphatidylinositol 3-kinase/Akt-dependent pathway. J Biol Chem 2004; 279: 4479-4489.*

21. *Kim DI, Lee TK, Lim IS, et al: Regulation of IGF-I production and proliferation of human leiomyomal smooth muscle cells by Scutellaria barbata D. Don in vitro: isolation of flavonoids of apigenin and luteolin as acting compounds. Toxicol Appl Pharmacol 2005; 205: 213-224.*

22. *Kim Jinh, Lee Eunok, Lee Hyoj, et al: Caspase Activation and Extracellular Signal-Regulated Kinase/Akt Inhibition Were Involved in Luteolin-Induced Apoptosis in Lewis Lung Carcinoma Cells. Ann N Y Acad Sci 2007; 1095: 598-611.*

23. *Zhang K, Guo QL, You QD, et al: Wogonin induces the granulocytic differentiation of human NB4 promyelocytic leukemia cells and up-regulates phospholipid scramblase 1 gene expression. Cancer Sci 2008; 99: 689-695.*

24. *Evan GI, Vousden KH: Proliferation, cell cycle and apoptosis in cancer. Nature 2001; 411: 342-348.*

25. *Dixon S, Soriano B, Lush R, et al: Apoptosis: its role in the development of malignancies and its potential as a novel therapeutic target. The Annals of pharmacotherapy 1997; 31: 76-82.*

26. *Parajuli P, Joshee N, Rimando AM, et al: In vitro antitumor mechanisms of various Scutellaria extracts and constituent flavonoids. 2009.*

27. *Hockenbery D, Nuñez G, Milliman C, et al: Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. 1990.*

28. *Ricci JE, Waterhouse N, Green DR: Mitochondrial functions during cell death, a complex (I-V) dilemma. Cell Death Differ 2003; 10: 488-492.*

29. *Reed JC, Green DR: Remodeling for demolition: changes in mitochondrial ultrastructure during apoptosis. Mol Cell 2002; 9: 1-3.*

30. *Wang F, Ma R, Yu L: Role of mitochondria and mitochondrial cytochrome c in tubeimoside I-mediated apoptosis of human cervical carcinoma HeLa cell line. Cancer Chemother Pharmacol 2006; 57: 389-399.*

31. *Gardner CR: Anticancer drug development based on modulation of the Bcl-2 family core apoptosis mechanism. Expert Rev Anticancer Ther 2004; 4: 1157-1177.*

32. *Kim KW, Jin UH, Kim DI, et al: Antiproliferative effect of Scutellaria barbata D. Don. on cultured human uterine leiomyoma cells by down-regulation of the expression of Bcl-2 protein. Phytother Res 2008; 22: 583-590.*

33. *Chui CH, Lau FY, Tang JC, et al: Activities of fresh juice of Scutellaria barbata and warmed water extract of Radix Sophorae Tonkinensis on anti-proliferation and apoptosis of human cancer cell lines. Int J Mol Med 2005; 16: 337-341.*

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# The relationship of the glycosylated hemoglobin A1c levels with the severity of the coronary artery disease in non-diabetic stable angina patients

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**Abstract**

**Objective:** We sought to determine the relationship between the severity of the coronary artery disease measured with the Gensini score and the hemoglobin A1c (HbA1c) levels in non-diabetic patients with stable angina pectoris.

**Methods:** A total of 93 patients undergoing coronary angiography were included in the study. Patients were divided into 3 groups by use of Gensini score (21 patients with normal coronary arteries, 26 patients with mild atherosclerosis and 46 patients with severe atherosclerotic lesions). The associations between severity of coronary artery disease and HbA1c levels were assessed using logistic regression analysis.

**Results:** The blood glucose readings were observed to be comparable between the groups ($p = 0.097$). While the HbA1c values were higher in severe atherosclerosis group compared with mild atherosclerosis and normal coronary arteries groups ($6.7 \pm 1.5$, $6.0 \pm 0.8$ and $5.6 \pm 0.6$%, respectively, $p = 0.002$). The HbA1c values were observed to be correlated with the Gensini score ($r = 0.374$, $p < 0.001$). A cutoff value of 6.0% for HbA1c predicted severe atherosclerosis with a sensitivity and specificity of 54% and 74%, respectively. In the multivariate analysis, high levels of HbA1c were observed to be independent predictors of severe atherosclerosis (OR: 1.975; 95% CI: 1.101-3.542, $p = 0.022$).

**Conclusion:** Increasing levels of HbA1c in non-diabetic patients with stable angina pectoris are associated with the severe atherosclerosis that may help to predict the increased risk for coronary artery disease.

**Key words:** Coronary artery disease, diabetes mellitus, HbA1c, Gensini.

## Introduction

Diabetes mellitus is a condition regarded to be equivalent to the coronary artery disease (1). Diabetes mellitus has also been found to be related with increased rates of cardiovascular morbidity and mortality (2). The hemoglobin A1c (HbA1c) levels are an indicator of the average blood glucose concentrations over a period of 2 to 3 months preceding the measurement (3). Therefore, this reading is considered to be a more reliable marker for the evaluation of the effects of long-term glycaemia in comparison to fasting glucose (4).

Blood glucose or glycosylated hemoglobin concentrations have been demonstrated to be indicators of the risk for the development diabetic complications (5). Also, higher fasting blood glucose levels with the normal range and higher HbA1c values in patients without diabetes mellitus have been demonstrated to be risk factors for cardiovascular events and subclinical atherosclerosis (6,7). Studies have shown that increased HbA1c levels are associated with increased rates of cardiovascular disease and all cause mortality both in diabetic and non-diabetic patients (8,9).

There are a number of studies focussing on the relationship between HbA1c levels and the presence and severity of the coronary artery disease (10-13). The majority of these studies have been conducted on patient groups with diabetes and acute coronary syndrome. Studies focussing on the relationship between the HbA1c levels and the Gensini score - which is a measure of the prevalence of the coronary artery disease - in non-diabetic patients with stable angina pectoris are limited in number. In this study, our aim is to investigate the relationship between the severity of the coronary artery disease measured with the Gensini score and the HbA1c values in non-diabetic patients with stable angina pectoris.

## Materials and methods

### *Study population*

For the purposes of the study, 93 consecutive patients who had undergone coronary angiographies in the Cardiology Clinic were enrolled. The study protocol has been approved by the local ethics committee and informed consent forms were read to all the patients before signing. Patients with acute coronary syndrome, diabetes mellitus, acute infectious diseases, chronic kidney and/or liver disease, and those with active malignancies and manifest heart failure were excluded from the study.

Detailed patient histories were obtained from all the patients and they all underwent complete physical examinations. The cardiac risk factors of the patients were recorded. Hypertension was defined as antihypertensive treatment or a blood pressure of > 140/90 mmHg. Diabetes mellitus was defined as a previous diagnosis of diabetes or use of antidiabetic medication. Active smokers were categorized as smokers. Coronary artery disease was based on family history, coronary artery disease in the first degree relatives before the age of 55 for males and before the age of 65 for females, or sudden death.

### *Coronary angiography and coronary artery disease*

All patients underwent standard selective coronary angiographies through the femoral artery using the Judkins technique. The images obtained through the coronary angiography were visually evaluated by two experienced interventional cardiologists independent from the study. Coronary artery disease was defined as ≥ 50% stenosis in at least one of the major epicardial coronary arteries (14).

The extent and severity of the coronary artery disease was evaluated through the Gensini score (15). The Gensini score was obtained by multiplying the Gensini severity coefficient determined for the percentage of the coronary artery stenosis (25%, 50%, 75%, 90%, 99% and full obstruction; Gensini scores: 1, 2, 4, 8, 16 and 32, respectively) with the coefficient varying between 0.5 and 5, which is determined according to the functional importance of the myocardial area nourished by the artery with the stenosis. The total Gensini score was reached by adding the scores obtained for each lesion.

The patients were divided into three groups as follows: Gensini score = 0 (normal coronary arteries), Gensini score < 30 (mild atherosclerosis), and Gensini score ≥ 30 (severe atherosclerosis) (16).

### *Laboratory tests*

Following a 10 to 12 hour fasting period before the coronary angiography, venous blood samples were obtained from the patients and were analysed in the laboratory of our hospital in terms of the total and high-density lipoprotein (HDL) cholesterol, triglycerides, glucose, urea, creatinine and complete blood count. Low-density lipoprotein cholesterol (LDL) was calculated using the Friedewald formula. The HbA1c analysis was performed using an HPLC system (Agilent Technologies, Palo Alto, USA) with columns for the HbA1c analysis (Recipe Chemicals, Munich, Germany).

### *Statistical analysis*

The statistical analyses were carried out using the SPSS 15.0 software. Parametric variables were expresses as mean ± standard deviation, while the categorical variables were expressed in percentage. The normality of the distribution of the data was tested through the Kolmogorov-Smirnov test. The continuous variables outside the normal distribution were analysed using the Mann-Whitney U-test, while those within the normal distribution were analysed through Student's t-test. The chi-square test was used for the intra-group comparison of the categorical variables. Spearman's rho test was used for the correlation analyses. The one-way analysis of variance (ANOVA) test was used to compare the three groups. Receiver operating characteristic (ROC) curve analysis was used to determine the optimum cut-off levels of the HbA1c in order to predict the severity of the atherosclerosis. The binary logistic regression analysis including the variables with a p value below 0.1 was carried out to identify the independent predictors of severe atherosclerosis and the results are expressed in odds ratio (OR) with a 95% confidence interval (CI). Statistical significance was based on a value of $P < 0.05$.

## Results

The study groups included 21 patients with normal coronary arteries (mean age: 58.4 ± 13.8 years), 26 patients with mild atherosclerosis (mean age: 60.5 ± 12.1 years), and 46 patients with severe atherosclerotic lesions (mean age: 63.0 ± 13.3 years) according to the results of the coronary angiography. The demographic and clinical features and the laboratory results of the patients are compared in Table 1.

No statistically significant difference has been observed among the groups in terms of the age, sex and the coronary risk factors. In the group with serious atherosclerosis, the HDL was observed to be lower (p = 0.021), while the other lipid parameters were similar. The blood glucose readings were observed to be comparable between the groups (p = 0.097). While the HbA1c values were found to be similar between the patient group with normal coronary arteries (5.6 ± 0.6%) and the group with mild atherosclerosis (6.0 ± 0.8%), these values were higher in the patient group with severe atherosclerosis (6.7 ± 1.5%) (p = 0.002, Figure 1).

The HbA1c values were observed to be correlated with the Gensini score (r = 0.374, p < 0.001), age (r = 0.266, p = 0.010), fasting blood glucose (r = 0.334, p = 0.001) and HDL (r = -0.359, p < 0.001). In the ROC analysis, when a value of 6.0% was set as the limit for the HbA1c, the presence of severe atherosclerosis was determined with 54% sensitivity and 74% specificity (area under the ROC curve: 0.677; 95% CI: 0.567-0.787; Figure 2). In the multivariate analysis, only high levels of HbA1c were observed to be independent predictors of severe atherosclerosis (OR: 1.975; 95% CI: 1.101-3.542, p = 0 .022; Table 2).

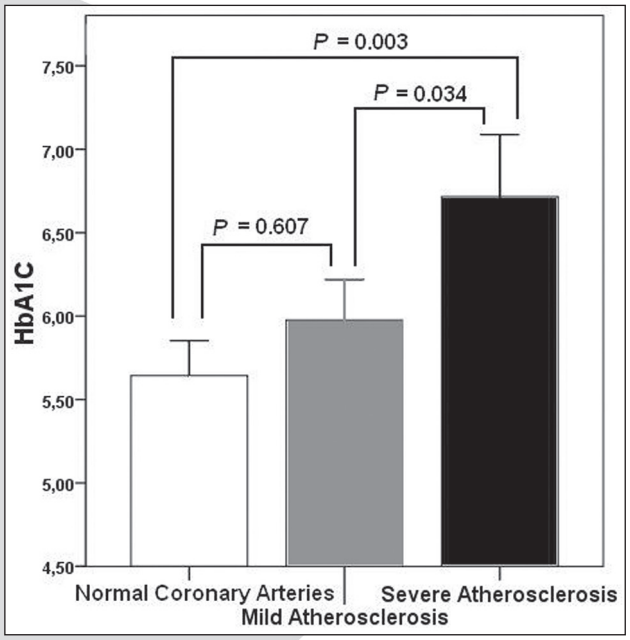


*Figure 1. Box plot of HbA1c levels in patients with normal coronary arteries, mild atherosclerosis and severe atherosclerosis.*

*Table 1. Baseline characteristics of the study population*

| | Normal coronary arteries (n = 21) | Mild atherosclerosis (n = 26) | Severe atherosclerosis (n = 46) | p |
|---|---|---|---|---|
| Age [years] | 58.4 ± 13.8 | 60.5 ± 12.1 | 63.0 ± 13.3 | 0.389 |
| Male patients | 9 (43%) | 20 (77%) | 30 (65%) | 0.051 |
| Coronary risk factors | | | | |
| Family history of CAD | 4 (22%) | 3 (10%) | 8 (17%) | 0.531 |
| Smoking | 6 (33%) | 10 (35%) | 18 (39%) | 0.876 |
| Arterial hypertension | 6 (33%) | 9 (31%) | 19 (41%) | 0.635 |
| Biochemical parameters | | | | |
| Glucose [mg/dl] | 109.0 ± 21.1 | 114.6 ± 24.2 | 122.4 ± 25.6 | 0.097 |
| Creatinine [mg/dl] | 0.8 ± 0.2 | 0.9 ± 0.3 | 1.0 ± 0.5 | 0.056 |
| Total cholesterol [mg/dl] | 183.6 ± 44.0 | 172.1 ± 45.8 | 163.0 ± 37.9 | 0.181 |
| LDL [mg/dl] | 107.7 ± 34.3 | 104.3 ± 34.9 | 97.0 ± 29.5 | 0.403 |
| HDL [mg/dl] | 41.4 ± 9.2 | 36.9 ± 11.4 | 34.1 ± 8.5 | **0.021** |
| Triglycerid [mg/dl] | 171.4 ± 80.2 | 159.0 ± 92.2 | 142.2 ± 81.1 | 0.401 |
| HbA1c (%) | 5.6 ± 0.6 | 6.0 ± 0.8 | 6.7 ± 1.5 | **0.002** |

*Mean values ± standard deviation and n (%) are reported for continous and categorical variables, respectively.*
*CAD, coronary artery disease; LDL, low density lipoprotein; HDL, high density lipoprotein; HbA1c, hemoglobin A1c.*

*Table 2. Multivariate logistic regression analysis to assess predictors of severe atherosclerosis*

| | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | OR (95% CI) | p | OR (95% CI) | p |
| Gender [male] | 0.859 (0.369-2.000) | 0.725 | 0.743 (0.272-2.026) | 0.561 |
| Glucose [mg/dl] | 1.018 (1.000-1.036) | 0.048 | 0.997 (0.974-1.021) | 0.822 |
| Creatinine [mg/dl] | 3.736 (0.902-15.478) | 0.069 | 2.807 (0.620-12.710) | 0.180 |
| HDL [mg/dl] | 0.950 (0.908-0.994) | 0.025 | 0.972 (0.924-1.023) | 0.275 |
| HbA1c (%) | 2.163 (1.305-3.587) | 0.003 | 1.975 (1.101-3.542) | **0.022** |

*CI, confidence interval; HDL, high density lipoprotein; HbA1c, hemoglobin A1c.*

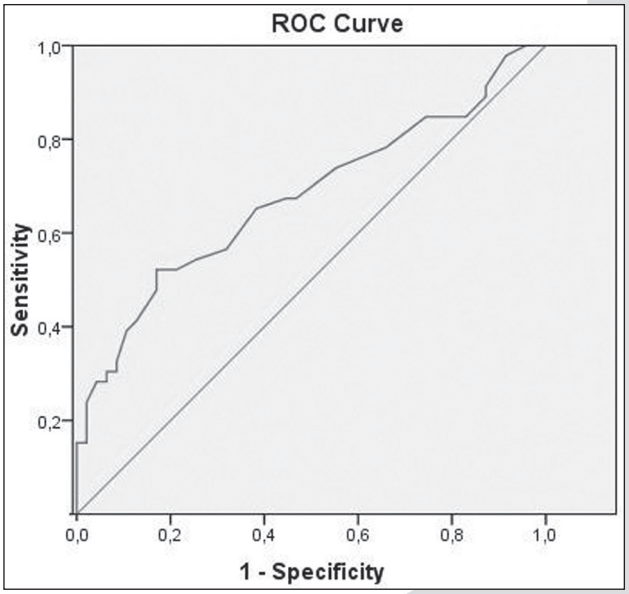


*Figure 2. ROC curve analysis of HbA1c levels predicting severity of coronary artery disease.*

## Discussion

This study has demonstrated that high HbA1c levels in non-diabetic patients with stable angina pectoris are an independent predictor of the severity of the coronary artery disease. In our study, the specificity of high HbA1c levels (>6%) in predicting the severity of the coronary artery disease was 74%, while its sensitivity was approximately 54%. Also, high HbA1c values were found to be correlated with the Gensini score.

HbA1c is a molecule with little biological variability and unaffected by the momentary changes in the glucose levels. It can be measured through a standardised method and without needing a fasting period before the measurement (17). Since HbA1c reflects the average glycemic status within the past 3 months (3), it is a more reliable marker compared to fasting blood glucose for the observation of the changes in the arterial walls brought about by long term glycaemia (4).

HbA1c levels are also more useful in predicting the abnormal glucose metabolism in comparison to the blood glucose levels on presentation in patients with coronary artery disease (18). Studies have shown that increased HbA1c levels are associated with increases in cardiovascular disease and all mortality both in diabetic and non-diabetic patients (8,9). Thus, an increase of 1% in the absolute glycosylated haemoglobin concentrations has been observed to lead to approximately 10–20% increase in the risk of cardiovascular diseases (8). Increased levels of HbA1c have been associated with increased mortality in the short and long term in the patients hospitalised due to coronary artery disease (3).

HbA1c levels have already been demonstrated to be associated with the severity of the coronary artery disease. In the study they conducted using multisliced computed tomography on asymptomatic and non-diabetic patients, Rivera et al. (19) have shown the relationship of increased HbA1c levels with the presence of coronary atherosclerosis and especially the coronary artery plaque load. Corpus et al. (20) have demonstrated that higher HbA1c levels are associated with a negative prognosis in a 12-month follow-up period in non-diabetic patients who undergo elective percutaneous coronary interventions. In their study conducted using the OGTT test on the patients who have undergone elective coronary angiographies, Li X et al. (12) have observed higher rates of multi-vessel lesions and increased severity of the stenosis in diabetic patients. Konstantinou et al. (4) have demonstrated that higher HbA1c levels in patients who had presented with chest pain and undergone coronary angiographies had predictive value in terms of the coronary artery disease. It is not astonishing that

more severe coronary artery disease is observed in patients with abnormal glucose metabolism. In our study, which included non-diabetic patients in contrast to these studies, increased HbA1c levels were found to be associated with higher Gensini scores indicating the severity of the atherosclerosis.

In a study they conducted on diabetic patients who had undergone coronary angiographies, Ravipati et al. (10) have observed that higher HbA1c levels were related to multi-vessel disease. In another study conducted on diabetic patients by Su et al. (13), the HbA1c levels were shown to be independent predictors of the Gensini score.

Some studies have investigated the HbA1c levels in patients with acute coronary syndrome. In a study by Saleem et al. (11) conducted in patients who applied with acute myocardial infarction, the increase in HbA1c was demonstrated to be an independent predictor of the Gensini score. In a study conducted in patients who had undergone primary percutaneous interventions due to acute myocardial infarction, Cicek et al. (21) have demonstrated increased HbA1c levels to be independent predictors of hospital mortality. Both of these studies were conducted in patients with acute myocardial infarction, whereas our patient population had different characteristics.

In the study by Lu et al. (22) where patients with diabetes mellitus who had normal coronary arteries were compared to the patients with severe coronary artery disease, no difference was observed in terms of the HbA1c readings. Hadjadj et al. (23) did not observe any relationship between the HbA1c levels and the prognosis of the patients who had myocardial infarctions. Timmer et al. (24) have demonstrated that increased HbA1c levels are not independent predictors of mortality in patients with ST elevation or non-ST elevation myocardial infarctions and with atypical chest pain. Although these studies did not point out any significant relationship between the HbA1c levels and atherosclerosis, it is observed that the patient groups in these studies are either heterogeneous, or small in number. Also, since in the study by Lu et al. (22) the diagnosis of the coronary artery disease was based on more severe coronary artery stenosis (>70%), a number of the patients may have been classified into the group without coronary artery disease and this may have influenced the end results.

***Study limitations***

The relatively small number of the study subjects constitutes the main limitation of this cross-sectional study. Since our study population consisted of a select group of patients referred for coronary angiography, it is not possible to extrapolate our results to the general population. Also, coronary angiography is not an ideal technique to quantify the overall atherosclerotic burden, since it only provides information about the arterial lumen. A third limitation results from the fact that the diagnosis of the diabetes mellitus was based on the clinical records and that the patients did not take oral glucose tolerance tests for the purposes of this study. This may have masked the true prevalence of the diabetes mellitus. Finally, the cross-sectional design of our study prevented the observation of any causal relationships between the HbA1c and the severity of coronary artery disease.

## Conclusion

The results obtained from the present study revealed that there is a correlation between the HbA1c levels and the Gensini score in non-diabetic patients with stable angina. Also, the HbA1c levels were observed to independently predict the severity of the coronary artery disease. Thus, in non-diabetic stable angina patients, the HbA1c levels may help to predict the increased risk for coronary artery disease. Further studies conducted on a larger patient groups are needed in order to confirm the association between the HbA1c and the coronary artery disease.

## References

1. *Singh S, Duggal J, Khosla N, Arora R. Screening guidelines for coronary heart disease in diabetes: current recommendations. J Cardiometab Syndr 2009; 4: 107-112.*

2. *Buse JB, Ginsberg HN, Bakris GL et al; American Heart Association; American Diabetes Association. Primary prevention of cardiovascular diseases in people with diabetes mellitus: a scientific statement from the American Heart Association and the American Diabetes Association. Circulation 2007; 115: 114-126.*

3. *Liu Y, Yang YM, Zhu J, Tan HQ, Liang Y, Li JD. Prognostic significance of hemoglobin A1c level in patients hospitalized with coronary artery disease. A systematic review and meta-analysis. Cardiovasc Diabetol 2011; 10: 98.*

4. *Konstantinou DM, Chatzizisis YS, Louridas GE, Parcharidis GE, Giannoglou GD. Non-diabetic hyperglycaemia correlates with angiographic coronary artery disease prevalence and severity. Diabetes Metab 2010; 36: 402-408.*

5. *Moss SE, Klein R, Klein BE, Meuer SM. The association of glycemia and cause-specific mortality in a diabetic population. Arch Intern Med 1994; 154: 2473-2479.*

6. *Coutinho M, Gerstein HC, Wang Y, Yusuf S. The relationship between glucose and incident cardiovascular events. A metaregression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. Diabetes Care 1999; 22: 233-240.*

7. *Nasir K, Santos RD, Tufail K et al. High-normal fasting blood glucose in non-diabetic range is associated with increased coronary artery calcium burden in asymptomatic men. Atherosclerosis 2007; 195: e155-160.*

8. *Khaw KT, Wareham N. Glycated hemoglobin as a marker of cardiovascular risk. Curr Opin Lipidol 2006; 17: 637-643.*

9. *Stratton IM, Adler AI, Neil HA et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ 2000; 321: 405-412.*

10. *Ravipati G, Aronow WS, Ahn C, Sujata K, Saulle LN, Weiss MB. Association of hemoglobin A(1c) level with the severity of coronary artery disease in patients with diabetes mellitus. Am J Cardiol 2006; 97: 968-969.*

11. *Saleem T, Mohammad KH, Abdel-Fattah MM, Abbasi AH. Association of glycosylated haemoglobin level and diabetes mellitus duration with the severity of coronary artery disease. Diab Vasc Dis Res 2008; 5: 184-189.*

12. *Li X, Gao X, Zhang B, Gu Q, Ren LM, Gao J. Glucose metabolism status and angiographic features of coronary artery in patients undergoing their first coronary angiography: study of 553 cases. Zhonghua Yi Xue Za Zhi 2006; 86: 1689-1692.*

13. *Su G, Mi S, Tao H et al. Association of glycemic variability and the presence and severity of coronary artery disease in patients with type 2 diabetes. Cardiovasc Diabetol 2011; 10: 19.*

14. *Ryan TJ, Faxon DP, Gunnar RM et al. Guidelines for percutaneous transluminal coronary angioplasty. A report of the American College of Cardiology/ American Heart Association Task Force on Assessment of Diagnostic and Therapeutic Cardiovascular Procedures (Subcommittee on Percutaneous Transluminal Coronary Angioplasty). Circulation 1988; 78: 486-502.*

15. *Gensini GG. A more meaningful scoring system for determining the severity of coronary heart disease. Am J Cardiol 1983; 51: 606.*

16. *Kaya H, Ertas F, Islamoglu Y et al. Association Between Neutrophil to Lymphocyte Ratio and Severity of Coronary Artery Disease. Clin Appl Thromb Hemost 2012 Jul 11. doi: 10.1177/1076029612452116.*

17. *American Diabetes Association. Standards of medical care in diabetes--2010. Diabetes Care 2010; 33: S11-61.*

18. *Ishihara M, Inoue I, Kawagoe T et al. Is admission hyperglycaemia in non-diabetic patients with acute myocardial infarction a surrogate for previously undiagnosed abnormal glucose tolerance? Eur Heart J 2006; 27: 2413-2419.*

19. *Rivera JJ, Choi EK, Yoon YE et al. Association between increasing levels of hemoglobin A1c and coronary atherosclerosis in asymptomatic individuals without diabetes mellitus. Coron Artery Dis 2010; 21: 157-163.*

20. *Corpus RA, O'Neill WW, Dixon SR, Timmis GC, Devlin WH. Relation of hemoglobin A1c to rate of major adverse cardiac events in nondiabetic patients undergoing percutaneous coronary revascularization. Am J Cardiol 2003; 92: 1282-1286.*

21. *Cicek G, Uyarel H, Ergelen M et al. Hemoglobin A1c as a prognostic marker in patients undergoing primary angioplasty for acute myocardial infarction. Coron Artery Dis 2011; 22: 131-137.*

22. *Lu L, Pu LJ, Xu XW et al. Association of serum levels of glycated albumin, C-reactive protein and tumor necrosis factor-alpha with the severity of coronary artery disease and renal impairment in patients with type 2 diabetes mellitus. Clin Biochem 2007; 40: 810-816.*

23. *Hadjadj S, Coisne D, Mauco G et al. Prognostic value of admission plasma glucose and HbA in acute myocardial infarction. Diabet Med 2004; 21: 305-310.*

24. *Timmer JR, Ottervanger JP, Bilo HJ et al. Prognostic value of admission glucose and glycosylated haemoglobin levels in acute coronary syndromes. QJM 2006; 99: 237-243.*

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# A clinical trial on post-thoracotomy pain management, Transdermal Fentanyl patch compared to intravenous Morphine

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**Abstract**

**Background:** Post thoracotomy analgesia has evolved with increasing knowledge about the impact of pain in recovery and new methods for treating it. Intravenous morphine commonly used to control moderate-to-severe postoperative pain. A transdermal system of Fentanyl delivery as an alternative treatment is recently approved. Transdermal Fentanyl patch is used to manage postoperative pain.

**Patients and methods:** We compared the effect of Transdermal Fentanyl patch versus Intravenous morphine morphine for patient's postoperative pain management under thoracotomy surgery. We conducted a randomized, prospective, multi central, clinical trial on fifty ASA class I-III patients scheduled for thoracic surgery. Patients were divided into two equal groups after the operation as followings: intra venous morphine (4 mg) was prescribed every 6 hours in the Morphine group, whereas in Fentanyl group a Transdermal Fentanyl patch (50µg/h) was placed one hour prior to anaesthesia. The anaesthetic method was the same in both groups. Pain score of patients was recorded based on visual analogue scale, facial expression type. An additional morphine dose was administered to the patients if needed. Analysis was done with T-test, Chi square test or Fisher's exact test.

**Results:** The efficacy of transdermal Fentanyl patch in 12, 18, 30, 48 hours post operation was better than the morphine group. The mean prescribed morphine dosage for the patients in Morphine group was 64±3.82 mg and in Fentanyl group 26±5.63 mg ($p< 0.05$).

**Conclusions:** In this study transdermal Fentanil patch showed to be an appropriate method instead of intermittent intra venous Morphine for postoperative analgesia and it is more effective in acute post thoracotomy pain controlling.

**Key words:** Transdermal Fentanyl patch, thoracotomy, Morphine, Pain, visual analogue scale.

## Introduction

Perioperative analgesia for thoracotomy has been evolved increasing knowledge about the impact of pain on recovery and new methods for treating it[1]. Thoracic surgery is one of the few areas where there is a general agreement among physicians regards to the importance of aggressive pain management[2]. Pain causes unwanted physiological and psychological complications, which have a great effect on the patients' prognosis[1-3]. Prevention of postoperative pain should be considered and managed as an important component that has a significant effect on patient outcome in terms of reducing oxygen consumption, $CO_2$ production and catecholamine release[2-4]. Post operation analgesia is an effective factor in the prognosis of thoracotomy surgery and could reduce lung complications. A wide range of analgesia methods have been reported to be effective in pain relief and it would be surprising if all have equal efficacy. [2-6].

Intermittent Patient-requested analgesia (PRN) allows patients to prescribe doses of opioids as needed to manage their post operation pain[7]. In PRN method, opioid analgesics are infused through an IV line and an extra dose would be prepared on patient demand. PRN have been used to maintain a mild level of pain rather than total pain relief, allowing the patient to be administered enough drugs to achieve a comfortable balance between

analgesia and adverse effects [8-10]. Problems that compromise patient safety, such as prescription errors, psycho-physical dependency and patient tampering have been reported [11]. To overcome these problems, trans-dermal Fentanyl patch is under development as an alternative method that delivers adequate doses of Fentanyl. Fentanyl is a lipid soluble synthetic opioid, which has been part of analgesic regimens for approximately 30 years. This drug is available in transdermal patch form, which is capable of continuous release of a determinate amount of Fentanyl per hour [6, 12, 13].

We designed this clinical trial to assess the efficacy of transdermal Fentanyl patch (TFP) as a narcotic pain-reliever in patient under thoracotomy surgery versus intermittent IV morphine for narcotic pain relief therapy. We assessed hemodynamic changes, incidence and severity of adverse events of TFP prescription besides a total assessment of pain control by TFP versus intermittent intravenous Morphine (PRN).

## Subjects and Methods

This clinical-trial study was approved by the local ethical committee of our university and a written informed consent was obtained from each patient. This study was registered in Iranian Registry of clinical trial (IRCT number: 201110107752N1).

Fifty patients with American Society of Anesthesiologist (ASA) class I-III with an age range of 22-83 years, scheduled for thoracic surgery under fixed method generalized anesthesia and surgery, were included in this prospective, multi central, randomized, double blind clinical trial.

Patients with history of pregnancy, long-term steroid therapy, dermatologic reactions or complications, allergy to the drugs, uncontrolled hypertension, dementia, neurologic or psychological disorders, patients undergoing intra pleural, intercostals blockage or epidural analgesia, alcohol abuse, renal insufficiency (serum creatinine more than 15 mg/dL) or liver dysfunction (aspartate aminotransferase, alanine aminotransferase or both more than 40 U/ L), preoperative respiratory function tests showing a forced vital capacity less than 60% predicted,1st second forced expiratory volume less than 60% or both, opium addiction or using any drug that modifies pain perception were excluded from the study.

Patients were allocated into two groups randomly. Before the operation, postoperative pain methodology was explained to all patients. Transdermal Fentanyl patch were prescribed to 25 patients (F group) and 25 patients were taken intra venous Morphine (M group) for analgesia.

Transdermal Fentanyl patch (Duragesic 50 μg/h) was placed on anterolateral side of patient's thorax skin one hour before induction of general anesthesia.

All patients kept NPO for about 8 hours before operation and received no premedication. Standard monitoring including ECG monitoring, capnograph, Non-invasive arterial blood pressure (NIBP), and pulseoximetry for peripheral oxygen saturation (SPO2) were applied for patients. After administration of Midazolam 0.03 mg/kg, fentanyl 1.5 μmg/kg and Morphine 0.1mg/kg, general anesthesia was induced with Thiopental 3-5mg/kg and Atracurium 0.05mg/kg. Anesthesia was maintained with Isoflorane 0.6-1.2% and N2O 50% and IV Fentanyl. Muscle relaxation was maintained with Atracurium, l0 mg IV, as required, and residual neuromuscular block was reversed with intra venous Neostigmine, 0.04 mg/kg, and Atropine, 0.02 mg/kg.

After thoracotomy, patients were transferred to ICU and monitored for 54 hours. The time of arrival at the ICU was considered as zero time.

The vital signs (VS) of patients included heart rate (HR), blood pressure (BP), respiratory rate (RR) and peripheral $O_2$ saturation were measured and recorded every six hours in ICU. If patient experienced reduced peripheral $O_2$ saturation and respiratory rate less than 8 per minute, IV Naloxone was administered to improve respiration status.

We used Morphine 4 mg IV every 6 hours after thoracotomy, in M group regardless of their pain score. Pain score of the patient was evaluated with facial expression type of Visual Analogue Scale (VAS) and recorded throughout the next 54 hours after thoracotomy in 2, 4, 6 and every 6 hours afterwards and a minute prior to IV Morphine [14,15]. An additional Morphine, 4 mg IV, was administered in both groups if the patient VAS was more than 2 or they needed additional analgesia. The amount of Morphine necessary for analgesia was compared in both groups at the end of the study. Degree of sedation was also examined by the same

observer on a five-point scale (0 = alert, 1 = mildly drowsy, 2 = moderately drowsy, easily rousable, 3 = very drowsy, rousable, 4 = difficult to rouse or 5 = unrousable) [16]. Side effects, including nausea, vomiting, respiratory depression, sedation and pruritus were recorded and treated with appropriate medication.

Pain classification was defined as below [14,15]:

0. No pain (VAS 0-2): neither pain complaint nor painful facial expression was observed, in clinical observation
1. Mild pain (VAS 3-4): In response to the question 'how are you', patient expressed a mild pain but it is tolerable.
2. Moderate pain (VAS 5-6): patient expressed pain and the patient's face was mildly clouded but it was tolerable with the explanation that this amount of pain is normal after any operation.
3. Severe pain (VAS 7-8): the patient expressed pain and it was not tolerable even with the explanation and patient's face was completely tense.
4. Worst imaginable pain (VAS 9-10): very severe and intolerable pain so that he asked others for help and the face was considerably tense as if crying.

Data were coded and entered into computer and analyzed by SPSS-16 software. Demographic data were studied. Continues covariates such as age and weight were compared using the analysis of variance T-test. For categorical covariates, the comparison was studied using a Chi-squared test or Fisher's exact test. For comparison of consecutive values, ANOVA and Repeated measurements Post Hoc test of variance were applied. The significance level was defined as a P value less than 0.05. To calculate the sample size, a power analysis of $\alpha$=0.05 and $\beta$=0.80 showed that 25 patients per study group were needed to detect the difference between two groups.

## Results

All patients (n=50) completed the study, 29 women and 21 men. The patients' age ranged from 22-83 years. There was no statistical difference in patients' demographics and mean duration of surgery between the two groups. Table 1

There was no significant difference between F and M groups on Post thoracotomy pain score in the 2nd, 4th hours but, According to what have been illustrated in the Table 2, there is a significant difference in Morphine requirements between two groups.

Figure 1 demonstrates better post operation pain management in 12th, 18th , 30th and 48th hours after thoracotomy in F group($p < 0.05$).

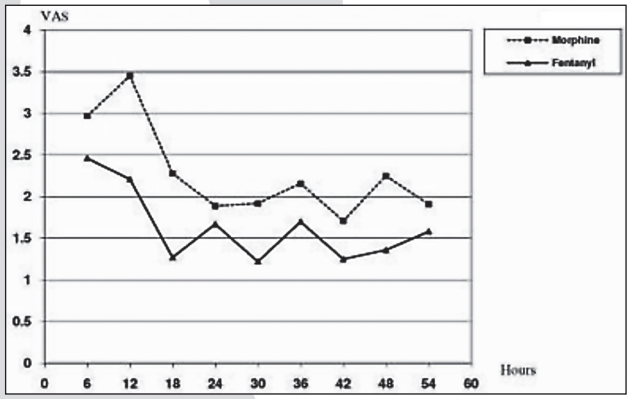


*Figure 1. Visual analogue scale comparison between the two groups (Fentanyle v.s Morphin) in the postoperative period*

*Table 1. Demographic data and duration of surgery*

| | Group F (n=25) | Group M (n=25) | P-value |
|---|---|---|---|
| Age (years) | 58.08±16.7 | 55.88±17.16 | 0.85 |
| Gender(male/female) | 14/11 | 13/12 | 0.62 |
| Weight(kg) | 56/4±6.6 | 62±7.2 | 0.74 |
| Surgical time (min) | 124±18 | 116±20 | 0.82 |

*Table 2. Mean VAS number (2nd, 4th h) and morphine requirement (mg) for each patient in two groups*

| Hours | 2nd h VAS | 4th h VAS | 6 | 12 | 18 | 24 | 30 | 36 | 42 | 48 | 54 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group M | 2.43±1.06 | 2.27±1.18 | 8.4±2.15 | 9.1±2.46 | 6.7±2.18 | 7.1±1.96 | 6.9±2.30 | 8.2±2.37 | 6±1.82 | 7.1±2.06 | 4.7±1.88 |
| Group F | 2.21±0.98 | 1.85±0.83 | 4.3±1.74 | 4.9±1.8 | 1.8±1.09 | 3.9±1.23 | 2.2±0.93 | 3.1±1.14 | 2.8±1.16 | 1.8±0.85 | 1.3±0.72 |
| p-value | 0.204 | 0.121 | 0.012 | 0.016 | 0.003 | 0.018 | 0.005 | 0.010 | 0.015 | 0.002 | 0.004 |

Figure 2 shows that morphine administration in F group was much less than M group and there was a statistically significant difference between the two groups in post operation duration.

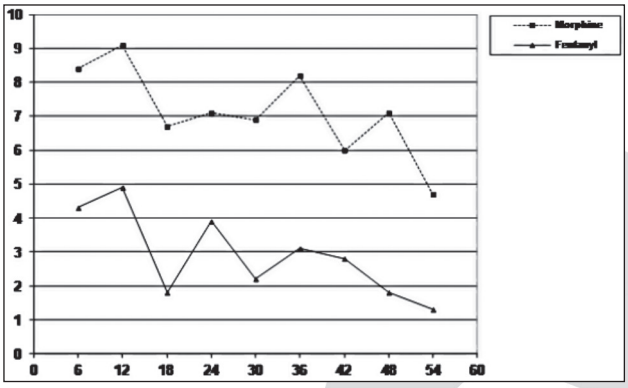


*Figure 2. Morphine requirement (mg) in the postoperative period*

The mean dose of morphine administration for each patient was significantly different between M and F group (64±3, 82 mg vs. 26±5.63 mg; P-value< 0.05). Total morphine administration in M group was 1605 mg and 655 mg in F group.

The mean amount of peripheral O2 saturation in each group was above 94% and no significant difference was observed. In addition, none of them experienced a respiratory rate less than 8 and there was no need for Naloxone administration. Moreover; during the study, all patients were hemodynamically stable and no opioid related hypotension or bradycardia was observed.

## Discussion

Post-thoracotomy pain is one of the most severe types of postoperative pain. The insufficient treatment of this pain results in reduced pulmonary compliance, atelectasis and pneumonia[17]. The opioid analgesic Fentanyl has a potential advantage over Morphine as it does not have active metabolites accumulate over time[18,19]. TFP had been used clinically since many years ago and has been effective in the management of chronic pain and various cancer-related pains[13, 20]. However, few studies have been performed on its effectiveness on acute post operation pain[21].

In our study, group F (TFP) had less Morphine administration and pain score, which was similar to other studies. In a randomized prospective study on 30 patients undergoing hip arthroplasty, patients were divided into two groups (N=15). The first group received TFP 10 hours prior to anesthetic induction whereas the second group received IV Morphine in a patient-controlled-analgesia method. Morphine administration and VAS in TFP group was considerably less than patient-controlled-analgesia method by IV Morphine group[21].

Another study performed by Dasgupta et al on 15 patients to examine the effectiveness of TFP on post thoracotomy pain management. They observed that TFP had decreased post operation pain score for 72 hours after the surgery and only nine patients needed supplement IV Pethedine[22]. In our study, we used Morphine as supplementary analgesia and in most of hours (until about 54) the pain management was better in patients who was administered TFP, and the amount of Morphine administration was lower.

The most common complications of TFP are nausea/vomiting and constipation but its most important complication is respiratory depression, which is more frequent in post operation (4%) than cancer related administration (2%). Respiratory complications in surgical patients mostly appear within the first 24 hours after the administration of Fentanyl patch[23]. Considering the 72 hours effect of Fentanyl, it is not recommended in those operations with shorter pain course, because it can cause decreased ventilation after relative pain control[24]. Our patients had been monitored for respiratory depression in ICU and no one experienced such complications.

TFP has several advantages: the total costs are less than intermittent IV morphine prescription, availability is like IV morphine, its use is easy and needs no expert nurse, it does not require IV access, risk of infection is low, and patient feels more comfortable; therefore TFP could be an interesting alternative. In addition, some researcher believe that, TFP have less side effects like constipation, nausea and vomiting and intolerance[7, 9,10,12,21].

In Rowbotham's study, patients were divided into 2 groups after upper abdomen surgery. The first group (22 patients) received transdermal Fentanyl two hours prior to the induction of anesthesia and the second group (18 patients) received placebo. Both groups received IV morphine as supplementary analgesia. Patients in Fentanyl group requested a considerable less amount of

morphine. Thus, authors suggested transdermal Fentanyl to manage acute post operation pain especially in surgeries, which have a severe and long postoperative pain [25]. We used IV Morphine for pain management and a better pain management in TFP group as well as a lower IV and supplementary Morphine administration was observed.

Ahmedzai et al reported that transdermal Fentanyl was the same as oral morphine in reduction of cancer pain[26]. Although in Allan's study, patients suffering from chronic low back pain experienced less pain in rest and sleep while using transdermal Fentanyl[27]. This may be due to continuous and stable plasma concentration of this form of the drug[23,28].

It should be mentioned that we had some strength and limitations in our study. Our studied group suffered from acute postoperative pain, which is different from the previous studied groups (chronic Pain). Patients using TFP have been experienced less for the acute postoperative pain management[29]. Although IV Morphine administered by a nurse based on the patients' request could be considered as a confounding factor because we did not have the patient-controlled analgesia facilities in our center. This could influence the analgesic effects of morphine via fluctuations in morphine plasma concentrations whereas, in Fentanyl group, the stability in Fentanyl plasma concentration has caused a better analgesia.

The results of our study suggest that transdermal Fentanyl patch is an appropriate and effective method for reduction of early post thoracotomy pain. TFP use is a safe and effective method that provides equal or better analgesia in comparison with intermittent intravenous morphine by patient's requested method (PRN).

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**References**

1. *Ochroch EA, Gottschalk A. Impact of acute pain and its management for thoracic surgical patients. 2005; 1: 105-21.*

2. *Gottschalk A, Cohen S, Yang S, Ochroch EA. Preventing and Treating Pain after Thoracic Surgery. Anesthesiology. 2006; 104: 594-600*

3. *Ong CKS, Lirk P, Seymour RA, Jenkins BJ. The Efficacy of Preemptive Analgesia for Acute Postoperative Pain Management: A Meta-Analysis. Anesth Analg 2005; 100: 757–73*

4. *Moiniche S, Kehlet H, Dahl JB. A Qualitative and Quantitative Systematic Review of Preemptive Analgesia for Postoperative Pain Relief: The Role of Timing of Analgesia. Anesthesiology 2002; 96: 725–41*

5. *Yegin A, Erdogan A, Kayacan N, Karsli B. Early postoperative pain management after thoracic surgery; pre- and postoperative versus postoperative epidural analgesia: a randomized study. European Journal of Cardio-thoracic Surgery 2003; 24: 420–424*

6. *Rathmell JP, Wu CL, Sinatra RS, Ballantyne JC, Ginsberg B,Gordon DB, Liu SS, Perkins FM, Reuben SS, Rosenquist RW, Viscusi ER. Acute post-surgical pain management: A critical appraisal of current practice. Reg Anesth Pain Med 2006; 31: 1-42.*

7. *Morad A, Winters B, StevensR, White E, Weingart J, Yaster M, et al. The Efficacy of Intravenous Patient-Controlled Analgesia After Intracranial Surgery of the Posterior Fossa: A Prospective, Randomized Controlled Trial Anesth Analg. 2012; 114: 416-423*

8. *Viscusi ER, Reynolds L, Chung F, Atkinson LE, Khanna S. Patient-controlled transdermal fentanyl hydrochloride vs intravenous morphine pump for postoperative pain: a randomized controlled trial. JAMA 2004; 291: 1333–41.*

9. *Chelly JE, Grass J, Houseman TW, Minkowitz H, Pue A. The safety and efficacy of a fentanyl patient-controlled transdermal system for acute postoperative analgesia: a multicenter, placebo-controlled trial. Anesth Analg 2004; 98: 427–33.*

10. *Howell PR, Pavy T, McMorland G, Douglas JM. Patient-controlled analgesia following caesarean section under general anesthesia: a comparison of fentanyl with morphine. Can J Anaesth. 1995; 42: 41-45.*

11. *Macintyre PE. Safety and efficacy of patient controlled analgesia. Br J Anaesth. 2001; 87: 36-46.*

12. *Gourlay G.K. Treatment of cancer pain with transdermal fentanyl. Lancet Oncol 2001; 2. 165-172.*

13. *Lukas Radbruch, Rainer Sabatowski, Frank Petzke, et al, Transdermal fentanyl for the management of cancer pain: a survey of 1005 patients. Palliative Medicine 2001; 15(4): 309-21*

14. *Chapman CR, Casey KL, Dubner R, Foley KM, Gracely RH, Reading AF. Pain measurement: an overview. Pain 2009; 22: 1–31.*

15. *De Boer AGEM, Van Lanschot JJB, Stalmeier PFM, Van Sandick JW, Hulscher JBF, De Haes CJM, et al .Is a single-item visual analogue scale as valid, reliable and responsive as multi-item scales in measuring quality of life? Quality of Life Research 2004; 13: 311–320.*

16. *Holdgate A, Asha S, Craig J, Thompson J Comparison of a verbal numeric rating scale with the visual analogue scale for the measurement of acute pain. Emergency Medicine 2003; 15: 441-446*

17. *Perttunen K, Nilsson E, Heinonen J. Exradural paravertebral and intercostal nerve blocks for postthoracotomy pain. Br J Anaesth 1995; 75: 541–7.*

18. *Guinard JP, Mavrocordatos P, Chiolero R, Carpenter RL. A randomized comparison of intravenous versus lumbar and thoracic epidural fentanyl for analgesia after thoracotomy. Anesthesiology 1992; 77: 1108–15.*

19. *Peng PWH, Sandler AN. A review of the use of fentanyl analgesia in the management of acute pain in adults. Anesthesiology. 1999; 90: 576-599.*

20. *Laurie Allan, Helen Hays, NielsHenrik Jensen,et al, Randomised crossover trial of transdermal fentanyl and sustained release oral morphine for treating chronic noncancer pain. BMJ 2001; 322: 1–7*

21. *Vincent Minville,Vincent Lubrano,Vincent Bounes,et al, Postoperative analgesia after total hiparthroplasty: patient-controlled analgesia versus transdermal fentanyl patch. Journal of Clinical Anesthesia 2008; 20: 280–283*

22. *Pereira B, Jain PN, Kakhandki V, Dasgupta D. Transdermal fentanyl in post-thoracotomy pain. JACP 1999; 15: 169-72.*

23. *Jeal W, Benfield P. Transdermal fentanyl: A review of its pharmacological properties and therapeutic efficacy in pain control. Drugs1997; 53: 109-38.*

24. *Kornick CA, Santiago-Palma J, Moryl N, Payne R, Obbens EA. Benefit-risk assessment of transdermal fentanyl for the treatment of chronic pain. Drug Saf 2003; 26: 951-73.*

25. *Rowbotham DJ, Wyld R, Peacock JE, Duthie DJ, Nimmo WS. Transdermal fentanyl for the relief of pain after upper abdominal surgery. Br J Anaesth 1989; 63: 56-9*

26. *Ahmedzai S, Brooks D. Transdermal fentanyl versus sustained-release oral morphine in cancer pain: preference, efficacy, and quality of life. The TTS-fentanyl comparative trial group.J Pain Symptom Manage 1997; 13: 254-61.*

27. *Allan L, Richarz U, Simpson K, Slappendel R. Transdermal fentanyl versus sustained release oral morphine in strong-opioid naïve patients with chronic low back pain. Spine 2005; 30: 2484-90.*

28. *Donner B, Zenz M, Tryba M, Strumpf M. Direct conversion from oral morphine to transdermal fentanyl: a multicenter study in patients with cancer pain. Pain 1996; 64: 527-534*

29. *Kim JG, Sohn SK, Kim DH, Baek JH, Chae YS, Bae NY, et al. Effectiveness of Transdermal Fentanyl Patch for Treatment of Acute Pain Due to Oral Mucositis in Patients Receiving Stem Cell Transplantation. Transplantation Proceedings. 2005; 37: 4488–4491*

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# Hydroxyurea may prolong survival of sickle cell patients by decreasing frequency of painful crises

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**Abstract**

**Background:** There may be some relationships between hydroxyurea treatment, frequency of painful crises, and survival of sickle cell diseases (SCDs) cases.

**Methods:** The study was performed in the Hematology Service on the SCDs cases. Frequency of painful crises and severity of them, as a mean degree between 0 to 10 according to patient's self-explanation, were detected. Cases with frequent crises, at least once a year, were put into the first, cases with rare crises into the second, and cases with no crisis in their lives were put into the third groups. Then, a hydroxyurea treatment was initiated to all patients with an initial dose of 15 mg/kg/day, and then it was increased up to 35 mg/kg/day according to patients' requirement and compliance.

**Results:** The study included 273 patients (135 females). Majority of them (79.1%) have frequent crises, 10.6% of them have rare crises, and 10.2% of them have no crisis in their lives before the initiation of treatment. There was an increase according to the mean ages from the first towards the third groups, progressively (28.0, 31.0, and 35.5 years), and the difference was highly significant between the first and third groups ($p<0.000$). The mean number (10.1 versus 1.3 crises per year, $p<0.000$) and severity of painful crises ((7.3 versus 2.4, $p<0.001$) were decreased with the hydroxyurea treatment, significantly.

**Conclusion:** Since the higher frequency of painful crises may indicate the shortened survival of the SCDs patients, hydroxyurea may prolong survival of such cases by decreasing frequency of painful crises, significantly.

**Key words:** Hydroxyurea, sickle cell diseases, painful crises, atherosclerosis.

## Introduction

Aging may be the major disease of the human being, and systemic atherosclerosis may be the major underlying cause of it. Atherosclerosis is an irreversible process that accelerated by many factors. Some of the accelerating factors are collected under the heading of metabolic syndrome including overweight, dyslipidemia, elevated blood pressure (BP), and insulin resistance for the development of terminal diseases such as obesity, hypertension (HT), diabetes mellitus (DM), coronary heart disease, chronic obstructive pulmonary disease (COPD), cirrhosis, chronic kidney disease (CKD), peripheric artery disease, and stroke (1-6). Probably sickle cell diseases (SCDs) are an accelerated systemic atherosclerotic process, too. They are characterized by sickle-shaped erythrocytes which is caused by homozygous inheritance of the hemoglobin S (Hb S). Glutamic acid is replaced with valine, as a less polar amino acid in the sixth position of the beta chain of the Hb S. Presence of the less polar amino acid promotes polymerisation of the Hb S. So Hb S causes erythrocytes to change their normal elastic and biconcave disc shaped structures to a hard and sickle shaped bodies. The decreased elasticity of erythrocytes is the central pathology of the disease. The sickling process is probably present in whole life, but it is exaggerated during various stressful conditions of the body. The erythrocytes can take their normal elastic shapes after normalization of the stressful conditions, but after repeated cycles of sickling and unsickling, they become a hard body, permanently. The sickled cells induced chronic endothelial damage and secondary tissue ischemia and infactions, even in the absence of an obvious vascular occlusion, are the final consequences of the disease, so life expectancy of the SCDs cases is

decreased by 25 to 30 years (7). We tried to understand whether or not there are some relationships between hydroxyurea treatment, frequency of painful crises, and survival of SCDs cases in the present study.

## Material and methods

The study was performed in the Hematology Service of the Mustafa Kemal University between March 2007 and December 2012. All patients with SCDs were enrolled into the study. SCDs are diagnosed by the hemoglobin electrophoresis performed via high performance liquid chromatography. Their medical histories including frequency and severity of painful crises, smoking habit, regular alcohol consumption, leg ulcers, and stroke were learnt. Frequency of painful crises was detected as a mean number of crises per year, and severity as a mean degree between 0 to 10 according to patient's self-explanation. Cases with a history of one pack-year were accepted as smokers, and cases with one alcoholic beverage a day were accepted as regular alcohol consumers. A check up procedure including serum creatinine level on three occasions, hepatic function tests, markers of hepatitis viruses A, B, and C and human immunodeficiency virus, an abdominal ultrasonography, a Doppler ultrasonography to evaluate the portal blood flow, an upper gastrointestinal tract endoscopy to detect esophageal varices just in suspected cases, and a computed tomography of the brain was performed. Cases with acute painful crises were treated at first, and then spirometric pulmonary function tests to diagnose COPD, the Doppler echocardiography to measure the systolic BP of pulmonary artery, and renal and hepatic function tests were performed on the silent phase. The criterion for diagnosis of COPD is post-bronchodilator forced expiratory volume in 1 second/forced vital capacity of less than 70% (8). Systolic BP of the pulmonary artery at and above 40mmHg on the silent phase is accepted as pulmonary hypertension (9). CKD is diagnosed with a continously elevated serum creatinine level which is greater than 1.2 mg/dL on the silent phase. Cases with renal transplantation were also put into the CKD group. Cirrhosis is diagnosed with hepatic function tests, ultrasonographic findings, esophageal varices, and ascites without histologic procedure in the absence of any indication. Digital clubbing is diagnosed by determining the ratio of distal phalangeal diameter to interphalangeal diameter which is required to be higher than 1.0, and with the presence of Swamroth sign (10,11). Cases with frequent crises, at least once a year, were put into the first, cases with rare crises were put into the second, and cases with no crisis in their lives were put into the third groups, and the groups were compared in between according to the mean ages. Then, a hydroxyurea treatment was initiated to all patients with an initial dose of 15 mg/kg/day, and then the dose was increased up to the final dose of 35 mg/kg/day according to patients' requirement and compliance. Finally, the mean numbers and severity of painful crises were detected in all cases after the hydroxyurea treatment, again, and compared with the previous values. Additionally, prevalences of smoking, regular alcohol consumption, pulmonary hypertension, leg ulcers, CKD, COPD, cirrhosis, digital clubbing, stroke, and exitus were detected among the patients. Mann-Whitney U test, Independent-Samples t test, and comparison of proportions were used as the methods of statistical analyses.

## Results

The study included 273 patients (138 males and 135 females). The mean age of them was 29.1 ± 9.4 (14-59) years. Majority of the SCDs patients (79.1%) have frequent crises, 10.6% of them have rare crises, and 10.2% of them have no crisis in their lives before the initiation of treatment (Table 1). When we compared the mean ages, there was an increase from the first towards the third groups, progressively (28.0, 31.0, and 35.5 years), and the difference was highly significant between the first and third groups ($p$<0.000). The hydroxyurea treatment was used and well-tolerated with a high majority of cases (91.5%), and the remaining cases could not been followed up. We have not observed any side effect of hydroxyurea therapy during the follow-up period. The final dose of 35 mg/kg/day was just achieved in 25 cases (9.1%), and the lowest terminal dose was 1.000 mg/day in two divided doses during the six-year follow-up period. During the period, the mean numbers of crises were significantly decreased with the treatment (10.1 versus 1.3 crises per

*Table 1. Frequency of painful crises in the sickle cell cases*

| Variables | Cases with frequent crises | p-value | Cases with rare crises | p-value | Cases without crisis | p-value* |
|---|---|---|---|---|---|---|
| Prevalence | 79.1% (216) | <0.001 | 10.6% (29) | ns† | 10.2% (28) | <0.001 |
| Mean age (year) | 28.0±8.9 (14-59) | ns | 31.0±9.8 (17-54) | ns | 35.5±10.3 (18-58) | 0.000 |

**Difference between the first and third groups †Nonsignificant (p>0.05)*

*Table 2. Characteristics of painful crises before and after hydroxyurea treatment*

| Variable | Before hydroxyurea treatment | p-value | After hydroxyurea treatment |
|---|---|---|---|
| Average number of crises (per year) | 10.1 ± 10.9 (1-48) | <0.000 | 1.3 ± 0.9 (0-4) |
| Average degree of severity of crises | 7.3 ± 2.5 (0-10) | <0.000 | 2.4 ± 1.9 (0-10) |

*Table 3. Associated pathologies of the sickle cell cases*

| Variables | Mean age (year) | Prevalence |
|---|---|---|
| Mortality | 28.7 ± 9.0 (19-45) | 5.4% (15) |
| Pulmonary hypertension | 30.4 ± 10.8 (19-56) | 11.3% (31) |
| Stroke | 31.6 ± 9.4 (17-47) | 4.7% (13) |
| Cirrhosis | 33.3 ± 11.7 (19-56) | 6.2% (17) |
| Chronic obstructive pulmonary disease | 34.0 ± 8.4 (23-54) | 6.5% (18) |
| Leg ulcers | 35.4 ± 7.4 (17-58) | 10.9% (30) |
| Digital clubbing | 35.5 ± 11.4 (21-56) | 5.8% (16) |
| Chronic kidney disease | 36.3 ± 9.9 (19-54) | 8.4% (23) |

year, $p<0.000$). Similarly, the mean severity of painful crises was also decreased with the treatment, significantly (7.3 versus 2.4, $p<0.001$). Although there was no patient with regular alcohol consumption, prevalence of smoking was high (9.5%). Because of the sistemic nature of the SCDs, we detected mortality (5.4%), pulmonary hypertension (11.3%), stroke (4.7%), cirrhosis (6.2%), COPD (6.5%), leg ulcers (10.9%), digital clubbing (5.8%), and CKD (8.4%) during the six-year follow-up period (Table 3). Five of the CKD cases were on hemodialysis, and one with renal transplantation. Histological diagnosis of cirrhosis was required in none of the study cases. Although antiHCV was positive in two of the cirrhotic cases, HCV RNA was detected as negative by polymerase chain reaction in both.

## Discussion

SCDs affect all vascular systems of the body (12-14). Even there was a patient with sickle cell retinopathy induced severe vision loss among our study cases. Eventually, the mean survival was 42 years for males and 48 years for females in the literature (7), whereas it was 26 and 31 years, respectively, in the present study. The great differences between the survival should be searched with further studies, but it may be secondary to the initiation of hydroxyurea treatment in early life in developed countries. On the other hand, the prolonged survival of females with SCDs should also be searched, effectively. As a result of such a great variety of clinical presentation, it is not surprising to see that the mean body weight and body mass index (BMI) were significantly retarded in the SCDs cases (15). On the other hand, as an opposite finding to some other reports (16,17), the mean body heights were nearly similar in the SCDs and control cases in the above study (15). Probably due to the significantly lower mean body weight and BMI, mean values of the low density lipoprotein cholesterol, alanine aminotransferase, and systolic and diastolic BPs were also significantly lower in the SCDs cases (15), which can be explained by definition of the metabolic syndrome (18-20).

Painful crises are the most common and disabling symptoms of the SCDs. Although some authors reported that painful crises themselves may not be

directly life threatening (21), infections are the most common precipitating factors of them. On the other hand, pain is the result of a complex and poorly understood interaction between erythrocytes, endothelial cells, leukocytes, and platelets at the moment. Whether leukocytosis contributes to the pathogenesis of the painful crises by releasing cytotoxic enzymes is unknown. For example, the adverse actions of neutrophils on endothelium are of particular interest with regard to the cerebrovascular diseases in SCDs. Leukocytosis even in the absence of any infection was an independent predictor of the severity of the disease (22), and it was associated with the risk of stroke in a cohort of Jamaican patients (23). Occlusions of vasculature of the bone marrow, bone infarctions, releasing of inflammatory mediators, and activation of afferent nerves may take role in the pathophysiology of the intolerable pain. Because of the severity of pain, narcotic analgesics are usually required to control them (24), but according to our practice, repeated erythrocyte transfusions may be highly significant in severe painful crises, both to relieve severe pain and to prevent sudden death that may develop secondary to acute chest syndrome or sepsis induced multiorgan failures on the chronic background of the SCDs.

Hydroxyurea is an effective therapeutic option used for the treatment of several disorders, such as chronic myeloproliferative disorders and SCDs. It interferes with cell division by blocking the formation of deoxyribonucleotides via inhibition of ribonucleotide reductase. The deoxyribonucleotides are the building blocks of DNA. Hydroxyurea mainly affects hyper-proliferating cells. Although the action way of hydroxyurea is thought to be the increase in gamma-globin synthesis for fetal hemoglobin (Hb F) in the SCDs (25,26), we think that its main action way is suppression of leukocytosis and thrombocytosis via blocking the DNA synthesis. By this way, the continuous inflammatory process of the SCDs that initiated at birth on the vascular endothelium is suppressed with some extent. Due to the same action way, hydroxyurea is also used in moderate and severe psoriasis to suppress hyper-proliferating skin cells. As in viral hepatitis cases, although presence of a continuous damage of sickled cells on the vascular endothelium, the severity of destructive process is probably exaggerated by the patient's immune system, especially by the actions of leukocytes and thrombocytes. So suppression of the hyper-proliferation of leukocytes and thrombocytes probably limits the endothelial damage-induced tissue ischemia and infarctions all over the body. Similarly, it was reported in a previous study that lower neutrophil counts were associated with lower crises rates, and if a tissue infarction occurs, lower neutrophil counts may limit severity of pain and extent of tissue damage (27). On the other hand, final Hb F levels in patients using hydroxyurea did not differ from their pretreatment levels, significantly (27).

Physicians at the National Institutes of Health Consensus Conference agreed that hydroxyurea is underused both in adults and children due to several reasons including the fact that hydroxyurea is a chemotherapeutic agent, and thus should not be taken by people planning to become pregnant in the near future, and the fear of a potentially increased risk of cancer (28). However, the cancer risk has not been substantiated by more than a decade of using hydroxyurea for adults (29). Although hydroxyurea is found in category D in pregnancy, and investigational and post-marketing data show risk to fetus (30), potential benefits may outweigh potential risk even in pregnancy, since it is becoming clear that untreated SCDs, even in the absence of any painful crisis, may terminate with several consequences even in the early adulthood (15). It is obvious that there is a need for more effective treatment options for SCDs, but until some better drugs become available, we have to use hydroxyurea in all SCDs cases, and its dose should be kept as higher in the moderate and severe SCDs patients.

Hydroxyurea probably has a life-saving role in the SCDs. As similar results to our study, the Multicenter Study of Hydroxyurea (MSH) studied 299 severely affected adults with sickle cell anemia (Hb SS), and compared the results in patients treated with hydroxyurea or a placebo (31). The study particularly searched effect of hydroxyurea on painful crises, acute chest syndrome, and requirement of blood transfusion. The outcomes were so overwhelming in the favour of hydroxyurea that the study was terminated after 22 months, and hydroxyurea was initiated for all patients (31). The MSH also demonstrated that patients treated with hydroxyurea had a 44% decrease in hospitalizations when compared with those taking placebo (31). In multi-

variable analyses, there was a strong and independent association of lower neutrophil counts with the lower crises rates (31). But this study was performed just in severe Hb SS cases alone, and the rate of painful crises was decreased from 4.5 to 2.5 per year (31). Whereas in our study, we used all kinds of SCDs cases with all clinical severity, and the rate of painful crises was decreased from 10.1 to 1.3 per year with an additional decreased severity of them, significantly (7.3 versus 2.4, $p<0.000$). In another study, adult patients using hydroxyurea for frequent painful crises appear to have reduced mortality after a 9-year follow-up period (32). The underlying disease severity remains critical to determine prognosis, but hydroxyurea may migitate severity of disease (32). Probably the chronic endothelial damage of the SCDs is initiated at birth and clinical complications of them begin in infancy. It was shown in another study that even infants with lower hemoglobin levels were more likely to have a higher incidence of clinical events such as acute chest syndrome, painful crises, and lower neuropsychological scores, and hydroxyurea reduced the incidence of them (33). Hydroxyurea in early life may also protect splenic function, improve growth, and prevent multiorgan dysfunctions by reversing early vascular damage. Transfusion programmes also reduce all of the complications of SCDs, however transfusions carry some risks including potential infection transmission, development of allo-antibodies causing subsequent transfusions more difficult, and iron overload. On the other hand, it has to be kept in mind that although hydroxyurea may minimize sickling-induced chronic endothelial damage and tissue ischemia and infarctions, the SCDs probably can not reversed completely, and painful crises, acute chest syndrome, autosplenectomy, and stroke may also occur during the treatment.

As a conclusion, since the higher frequency of painful crises may indicate the shortened survival of the SCDs cases, hydroxyurea may prolong survival of such patients by decreasing frequency of painful crises, significantly.

## References


1. *Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet 2005; 365: 1415-1428.*
2. *Helvaci MR, Kaya H, Gundogdu M. Association of increased triglyceride levels in metabolic syndrome with coronary artery disease. Pak J Med Sci 2010; 26: 667-672.*
3. *Helvaci MR, Kaya H, Borazan A, Ozer C, Seyhanli M, Yalcin A. Metformin and parameters of physical health. Intern Med 2008; 47: 697-703.*
4. *Helvaci MR, Kaya H, Seyhanli M, Yalcin A. White coat hypertension in definition of metabolic syndrome. Int Heart J 2008; 49: 449-457.*
5. *Helvaci MR, Kaya H, Seyhanli M, Cosar E. White coat hypertension is associated with a greater all-cause mortality. J Health Sci 2007; 53: 156-160.*
6. *Helvaci MR, Aydin LY, Aydin Y. Chronic obstructive pulmonary disease may be one of the terminal end points of metabolic syndrome. Pak J Med Sci 2012; 28: 376-379.*
7. *Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Steinberg MH, et al. Mortality in sickle cell disease. Life expectancy and risk factors for early death. N Engl J Med 1994; 330: 1639-1644.*
8. *Global strategy for the diagnosis, management and prevention of chronic obstructive pulmonary disease 2010. Global initiative for chronic obstructive lung disease (GOLD).*
9. *Fisher MR, Forfia PR, Chamera E, Housten-Harris T, Champion HC, Girgis RE, et al. Accuracy of Doppler echocardiography in the hemodynamic assessment of pulmonary hypertension. Am J Respir Crit Care Med 2009; 179: 615-621.*
10. *Schamroth L. Personal experience. S Afr Med J 1976; 50: 297-300.*
11. *Vandemergel X, Renneboog B. Prevalence, aetiologies and significance of clubbing in a department of general internal medicine. Eur J Intern Med 2008; 19: 325-329.*
12. *Haupt HM, Moore GW, Bauer TW, Hutchins GM. The lung in sickle cell disease. Chest 1982; 81: 332-337.*
13. *Shapiro MP, Hayes JA. Fat embolism in sickle cell disease. Report of a case with brief review of the literature. Arch Intern Med 1984; 144: 181-182.*

14. *Hutchinson RM, Merrick MV, White JM. Fat embolism in sickle cell disease. J Clin Pathol 1973; 26: 620-622.*

15. *Helvaci MR, Kaya H. Effect of sickle cell diseases on height and weight. Pak J Med Sci 2011; 27: 361-364.*

16. *Al-Saqladi AW, Cipolotti R, Fijnvandraat K, Brabin BJ. Growth and nutritional status of children with homozygous sickle cell disease. Ann Trop Paediatr 2008; 28: 165-189.*

17. *Zemel BS, Kawchak DA, Ohene-Frempong K, Schall JI, Stallings VA. Effects of delayed pubertal development, nutritional status, and disease severity on longitudinal patterns of growth failure in children with sickle cell disease. Pediatr Res 2007; 61: 607-613.*

18. *Helvaci MR, Kaya H, Yalcin A, Kuvandik G. Prevalence of white coat hypertension in underweight and overweight subjects. Int Heart J 2007; 48: 605-613.*

19. *Helvaci MR, Kaya H, Duru M, Yalcin A. What is the relationship between white coat hypertension and dyslipidemia? Int Heart J 2008; 49: 87-93.*

20. *Helvaci MR, Kaya H, Sevinc A, Camci C. Body weight and white coat hypertension. Pak J Med Sci 2009; 25: 6: 916-921.*

21. *Parfrey NA, Moore W, Hutchins GM. Is pain crisis a cause of death in sickle cell disease? Am J Clin Pathol 1985; 84: 209-212.*

22. *Miller ST, Sleeper LA, Pegelow CH, Enos LE, Wang WC, Weiner SJ, et al. Prediction of adverse outcomes in children with sickle cell disease. N Engl J Med 2000; 342: 83-89.*

23. *Balkaran B, Char G, Morris JS, Thomas PW, Serjeant BE, Serjeant GR. Stroke in a cohort of patients with homozygous sickle cell disease. J Pediatr 1992; 120: 360-366.*

24. *Cole TB, Sprinkle RH, Smith SJ, Buchanan GR. Intravenous narcotic therapy for children with severe sickle cell pain crisis. Am J Dis Child 1986; 140: 1255-1259.*

25. *Miller BA, Platt O, Hope S, Dover G, Nathan DG. Influence of hydroxyurea on fetal hemoglobin production in vitro. Blood 1987; 70: 1824-1829.*

26. *Platt OS. Is there treatment for sickle cell anemia? N Engl J Med 1988; 319: 1479-1480.*

27. *Charache S. Mechanism of action of hydroxyurea in the management of sickle cell anemia in adults. Semin Hematol 1997; 34: 15-21.*

28. *Brawley OW, Cornelius LJ, Edwards LR, Gamble VN, Green BL, Inturrisi CE, et al. NIH consensus development statement on hydroxyurea treatment for sickle cell disease. NIH Consens State Sci Statements 2008; 25: 1-30.*

29. *Tefferi A. Polycythemia vera and essential thrombocythemia: 2012 update on diagnosis, risk stratification, and management. Am J Hematol 2012; 87: 285-293.*

30. *Campion SN, Davenport SJ, Nowland WS, Cappon GD, Bowman CJ, Hurtt ME. Sensitive windows of skeletal development in rabbits determined by hydroxyurea exposure at different times throughout gestation. Birth Defects Res B Dev Reprod Toxicol 2012; 95: 238-249.*

31. *Charache S, Barton FB, Moore RD, Terrin ML, Steinberg MH, Dover GJ, et al. Hydroxyurea and sickle cell anemia. Clinical utility of a myelosuppressive "switching" agent. The Multicenter Study of Hydroxyurea in Sickle Cell Anemia. Medicine (Baltimore) 1996; 75: 300-326.*

32. *Steinberg MH, Barton F, Castro O, Pegelow CH, Ballas SK, Kutlar A, et al. Effect of hydroxyurea on mortality and morbidity in adult sickle cell anemia: risks and benefits up to 9 years of treatment. JAMA 2003; 289: 1645-1651.*

33. *Lebensburger JD, Miller ST, Howard TH, Casella JF, Brown RC, Lu M, et al; BABY HUG Investigators. Influence of severity of anemia on clinical findings in infants with sickle cell anemia: analyses from the BABY HUG study. Pediatr Blood Cancer 2012; 59: 675-678.*

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# The influence of threshold value variations on the 3D reconstruction of tooth structures based on cone beam computed tomography – ex vivo study

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**Abstract**

**Aim:** The aim of this *ex vivo* study was to investigate the influence of threshold value variation on the three-dimensional reconstructions of external and internal dental structures based on cone-beam computed tomography (CBCT) data.

**Methodology:** Two human teeth: one upper canine and one first upper molar were scanned by ProMax 3D (voxel size 160 μm). Data were processed using MIMICS software and based on particular thresholding results, the set of appropriate three-dimensional reconstructions was created. The levels of lower threshold were established at 400, 800, 1100, 1300, 1500 HU for canine and, additionally, 1700 HU for molar. For the purpose of quantitative assessment the volume and some overall dimensions of dental tissues as well as the dimensions of particular roots (external dimensions) and canals (internal dimensions) were determined.

**Results:** Provided 3D reconstructions revealed many anatomical details, e.g. canal splitting in the canine and extensive anastomosis between MB and MB2 canals, however, the quality of these details differed depending on the lower threshold value. Raised lower threshold value has resulted in shortened outer dimensions of individual models, as well as in enlarged pulp cavity. Thresholding process influenced obtained measurements and had significant impact on relation between canal dimensions and external root dimensions.

**Conclusions:** The value of the lower threshold influences the quality of reconstruction of objects examined by CBCT and the degree of visualization of details in the dental cavity structure. Therefore, the thresholding process should be carried out with special caution.

**Key words:** threshold value, 3D reconstruction, cone beam computed tomography.

## Introduction

The knowledge of root canal anatomy, with its many variations, is critical for the success of endodontic therapy. Conventional and digital intraoral radiographs are not completely reliable in assessing root canal morphology because of their main limitation connected with two-dimensional reconstruction of three-dimensional objects. It causes many distortions, artifacts and superimposition of dental structures on X-ray images. The tools which may help to avoid such problems are computed tomography techniques (1-2). In spite of the fact that conventional tomography can be useful in diagnosis of complicated endodontic cases, it is not widely used because of low spatial resolution compared to the dimension of root canals, high radiation dose as well as size and cost of the equipment (2). Microtomography (μCT) is considered as a reference standard in evaluation of tooth morphology because it offers much higher accuracy than CT. However, it can only be used *ex vivo* because of time-consuming procedures and high radiation dose, as well as limitation of the scanned object size (2-5).

Recently, the cone-beam computed tomography has become available for dental practice. Major benefits of this technology are in-office imagining, real size data, isotropic voxels, high resolution, relatively low radiation dose and the possibility to generate different kind of images (e.g. orthopantomogram, cefalogram, TMJ) as well as fewer disturbances from metal artifacts compared with CT. The CBCT scanner software allows carrying out a multi-planar reformation and 3D surface rendering of the anatomic structures; they can be studied from any spatial plane without superposition. Presently, CBCT is widely used for planning implants and in orthodontic treatment, presurgical planning, diagnosis of periapical pat-

hology, complicated endodontic treatment and diagnosis of traumatic injuries. This technology is considered a promising tool for endodontic practice which in future will help not only to identify details of root canal morphology, but also to select tools and techniques or to simulate clinical procedures (5-7). The accuracy of CBCT data of oral and maxillofacial region was widely evaluated, but the number of studies concerning root canal morphology is limited (5,6,8-12). Canal reconstructions provided with data from CBCT are more precise than those from multi-sliced CT (13).

The attenuation of X-radiation in computed tomography is measured in Hounsfield units (HU). The scale is linear, and the scope of measured values usually ranges from -1000 to +3000. Attenuation of water is 0, of air -1000 HU, and of a bone +400 HU and higher (14). Within a tooth, there are many structures with different radiation absorption coefficients: empty spaces (in tooth with open cavity), uncalcified tissues (pulp), calcified tissues (enamel, dentin, cementum) and obturation materials. Additionally, dentin is not a homogeneous tissue, as its structure near the pulp is irregular or dystrophic (3). Tomographic scans provide huge data amount, and a special technique called thresholding was developed to adapt eye perception to data analysis. This method consists in choosing threshold values which will limit the range of visualized tissues. At the CT devices, some thresholds are predefined, but, ultimately, determination of them is arbitrary and depends on an operator's interpretation (14). Thresholding could be used as a method for tooth segmentation into enamel, dentin and pulp space (4). The influence of threshold variations on the assessment of structural and mechanical properties of the bone was described in the literature, however, the number of studies taking into consideration their influence on teeth reconstructions is limited (15-17). No reports on the influence of threshold variations on the visualization of canal morphology with reference to data from cone-beam computed tomography were found.

The aim of this *ex vivo* study was to investigate the influence of threshold value variation on the three-dimensional reconstructions of external and internal dental structures based on cone-beam computed tomography data.

## Material and Methods

Two human teeth: one upper canine and one first upper molar were scanned by ProMax 3D® (Planmeca Oy, Helsinki, Finland). Teeth were fixed by foamed polystyrene to reduce artifacts from fixing material. The field of view was 80x80 mm and the settings on the ProMax 3D® were 70 kV and 10 mA. 501 layers with the resolution of 501x501 pixels were registered. The voxels were isotropic and their size was 160 μm. Obtained data were processed by MIMICS® (Materialise N.V., Leuven, Belgium) software. Three-dimensional reconstructions of both teeth were made according to threshold variations expressed in Hounsfield units. The levels of lower threshold were established at 400, 800, 1100, 1300, 1500 HU for canine and, additionally, 1700 HU for molar. The upper threshold was constant at 2976 HU. Based on particular thresholding results, the set of appropriate models presenting root canals and hard tissues was created. For better visualization of the dental hard tissues, they were presented as semitransparent. As a result, all places where two or more surfaces overlapped became opaque. In this way, the canal system was presented as well contrasting against the background of external tooth surface. To analyze morphology of the reconstructed canals system more easily, solid models for particular thresholding results were also created. For this purpose the empty spaces, occurring within particular teeth reconstructions volume after appropriate thresholding process, were filled in slice by slice. The new material layers filling these empty spaces were next combined as the solid model of root canal. The filling process was conducted only for these layers where the space of root canal was closed; it means it was fully surrounded by hard tissues. The exceptions were made for models of canine tooth obtained for values of lower threshold limit of 1100-1500 HU. Namely, in these cases in 1/3 apical region on lateral surfaces occurred discontinuities of the dentin layer which needed to be filled manually. The canal reconstructed in this way may slightly differ from the actual form. However, without carrying out such procedure, it would be impossible to reconstruct the canal in its apical part.

For the purpose of quantitative assessment of differences occurring among the reconstructions of examined teeth, the volume of hard dental ti-

ssues, the length of the canine and the lengths of individual roots of the molar were evaluated. Furthermore, the dimensions of individual roots (external dimensions) and canals (internal dimensions) were determined; the diagram of these measurements with the indications of individual dimensions is shown in Figure 1. An analysis for two randomly selected canine sections was carried out (a and b levels). In case of the molar, measurements in three sections were made; two of them (a and b levels) were randomly selected but common for all roots, whereas the third (c level) was determined in such a way that it was situated at a distance of 5 mm from the root apex. Obtained data was used to determine the internal/external ratio representing the relation of dental cavity dimensions to root dimensions and, in addition, the mean values and the standard deviation for the internal/external ratio were calculated.

## Results

### *Qualitative analysis*

Figure 2 shows three-dimensional models of pulp cavity of the examined canine in frontal and sagittal view made on the basis of CBCT data for particular values of the lower threshold. Depending on the set limit of the window, differences in the reconstruction of anatomical details, particularly in the 1/3 apical region, were visible. With the HU value of 400 the root canal was not entirely reconstructed in this part. An increase of the lower threshold limit caused consistent lengthening of the canal. The 3D reconstruction in the sagittal section (Figure 2b) visualized the configuration of a type IV root canal according to Weine's classification, which was not visible in the frontal section (Figure 2a). Also in this section, the canal models differed depending on assumed lower threshold values from residual construction of the apical region with 400 HU to nearly complete connection of both canals with 1500 HU. On the other hand, figure 3 shows 3D reconstructions of both outer and inner structures of examined canine obtained from cone-beam tomography. In successive 3D models we noticed that while the pulp cavity is extended as a result of increased lower threshold values, a simultaneous loss of hard tissues in the root apex region occurred.

Figure 4 shows, in a view of the mesial surface, three-dimensional models of the pulp cavity of the first upper molar developed on the basis of CBCT data depending on the width of the window. Similarly as in case of the canine, the selected lower limit of the window had a significant influence on the shape of obtained model, which is best visible in relation to MB and MB2 canals. With the value amounting to 400 HU, the MB2 canal was almost invisible. Models made for lower threshold values amounting to 800-1300 HU visualized both this canal and an extensive anastomosis between MB and MB2 canals. On the other hand, in the pulp cavity model obtained for 1700 HU threshold, the MB and MB2 canals were completely fused. The

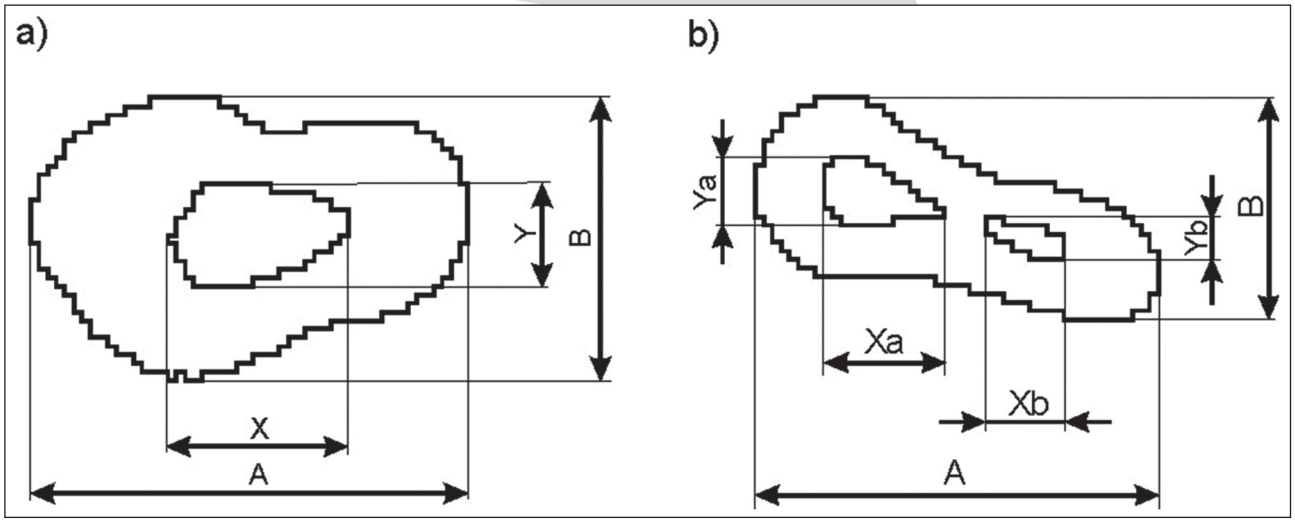


*Figure 1. The scheme of internal and external root dimensions: a) in case of the canine and the palatal and distobuccal roots of the molar, b) a diagram used for the mesiobuccal root of the molar.*

reconstructions of hard tissues and root canals of examined molar (Figure 5) show a similar tendency as in case of the canine, i.e. an increase of the lower window threshold limit caused the loss of hard tissues in the root apex region with simultaneous expansion of the pulp cavity.

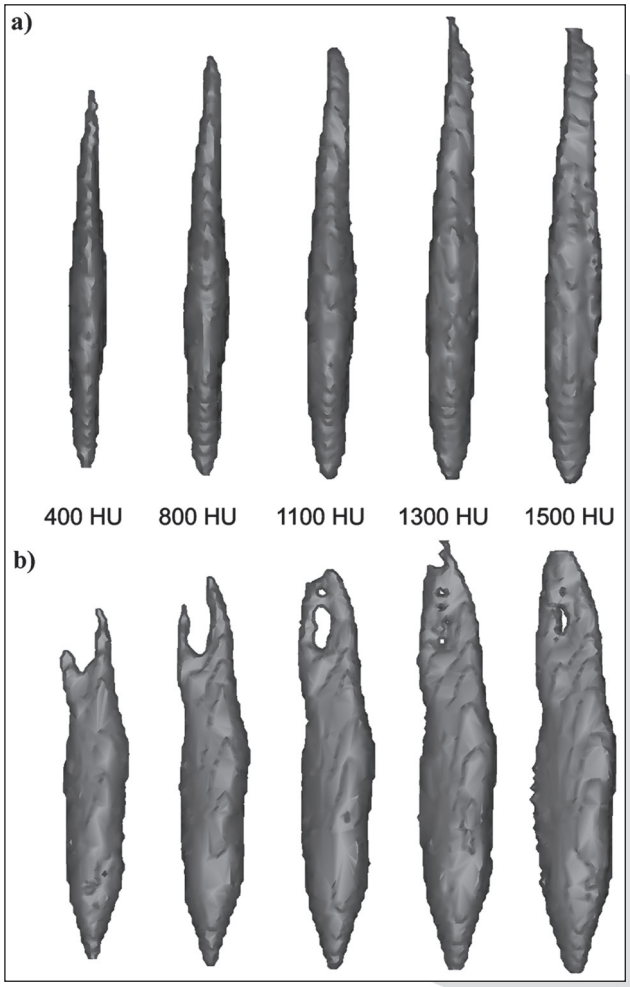


*Figure 2. Three-dimensional reconstruction of root canal system of the upper canine based on cone beam computed tomography data according to different threshold values: (a) frontal view, (b) sagittal view.*

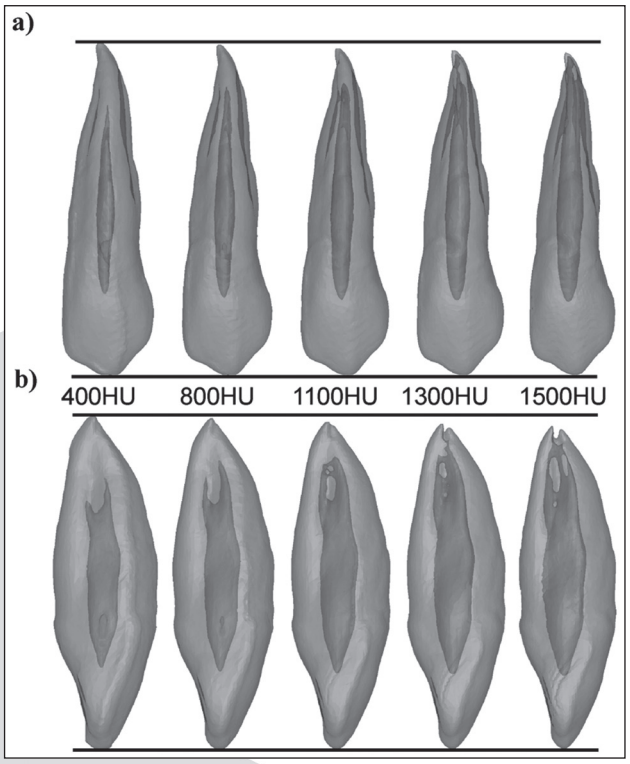


*Figure 3. Three-dimensional reconstruction of the hard tissues and root canal system of the upper canine based on cone beam computed tomography data according to different threshold values: (a) frontal view, (b) sagittal view.*

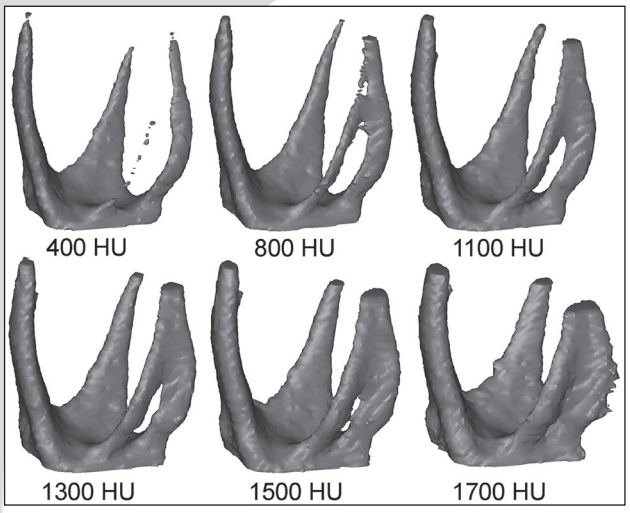


*Figure 4. Three-dimensional reconstruction of the root canals of the upper molar based on cone beam computed tomography data according to different threshold values, sagittal view.*

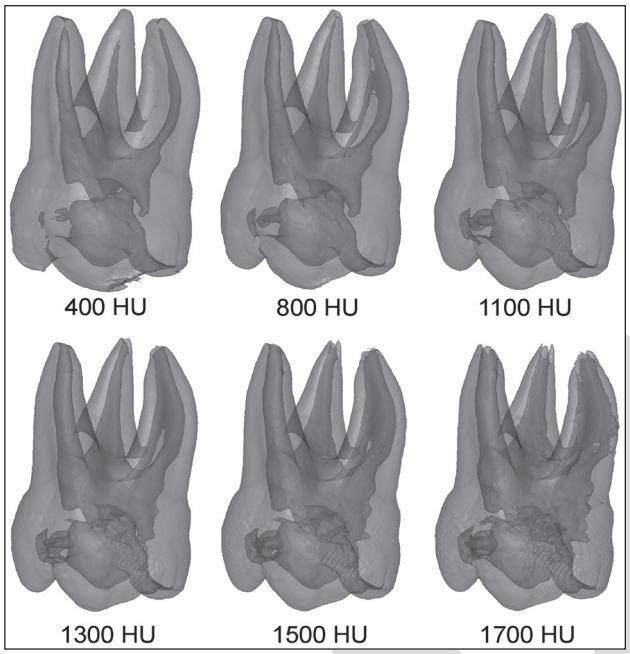


*Figure 5. Three-dimensional reconstruction of the hard tissues and root canal system of the upper molar based on cone beam computed tomography data according to different threshold values, sagittal view.*

***Quantitative analysis***

Table 1 shows the results of canine measurements. In the comparison of dimensions obtained for the adopted threshold values (400 HU and 1500 HU) the tooth length measured from the end of the incisal cusp to the root apex decreased by 0.8 mm, and the volume of hard tissues decreased by 163 $mm^3$ (24.8%). An analysis of external and internal dimensions of examined canine confirmed that with increasing lower threshold the root diameter decreases, and the canal diameter increases. This process is clearly illustrated in the internal/external ratio results. In addition, we observed that in the discussed case the mesial-distal root canal dimension (Y) was the most susceptible to diameter fluctuations; its diameter, measured at the B level, increased twofold.

Table 2 illustrates the results of an analysis carried out for the molar. The decrease in length of individual roots between the lower limit 400 HU and 1700 HU amounted to 0.46 mm for the palatal root, 0,56 mm for the mesiobuccal root and 0,8 mm for the distobuccal root, respectively. The difference in volume of hard dental tissues amounted to 388 mm3 (25.8%). For the sake of clarity of results shown in Table 2 the presentation of individual internal and external dimensions of individual roots was omitted, and only internal/external ratio values were specified. The results of the analysis clearly indicate that in case of the examined molar the thresholding process had a similar effect on the relations between external and internal dimensions as discussed

*Table 1. Canine measurement results*

| | **Lower threshold value** | | | | |
|---|---|---|---|---|---|
| | **400** | **800** | **1100** | **1300** | **1500** |
| **Length [mm]** | 27.68 | 27.52 | 27.2 | 27.04 | 26.88 |
| **Hard tissue volume [$mm^3$]** | 657 | 598 | 556 | 527 | 494 |
| **Dimensions at the A level [mm]** | | | | | |
| A | 8.64 | 8.48 | 8.16 | 8.16 | 8.16 |
| B | 5.76 | 5.44 | 5.44 | 5.28 | 5.12 |
| X | 2.72 | 3.04 | 3.04 | 3.36 | 3.68 |
| Y | 1.44 | 1.6 | 1.92 | 1.92 | 2.24 |
| **Internal/external dimension ratio at the A level** | | | | | |
| X/A | 0.315 | 0.358 | 0.373 | 0.412 | 0.451 |
| Y/B | 0.250 | 0.294 | 0.353 | 0.364 | 0.437 |
| **Dimensions at the B level [mm]** | | | | | |
| A | 7.36 | 7.20 | 7.20 | 6.88 | 6.88 |
| B | 4.48 | 4.32 | 4.16 | 4.00 | 4.00 |
| X | 2.24 | 2.4 | 2.56 | 2.88 | 2.88 |
| Y | 0.8 | 1.12 | 1.28 | 1.44 | 1.6 |
| **Internal/external dimension ratio at the B level** | | | | | |
| X/A | 0.304 | 0.333 | 0.356 | 0.419 | 0.419 |
| Y/B | 0.179 | 0.259 | 0.308 | 0.360 | 0.400 |

in case of the canine. Noteworthy is the fact that in case of the mesiobuccal root, within a certain length, the division of the canal into two separate branches occurred. The reproduction of this phenomenon in a model depends to a considerable degree on the adopted lower threshold level, which has slightly complicated the calculations made for this root (X=Xa+Xb; Y=Ya+Yb), and the results obtained in this way were characterized by a slightly greater scatter. In view of the differences in analysis methodology, they were not taken into consideration in the statistical analysis the results of which were shown in Figure 6. Within the scope of the analysis, the mean value and the standard deviation for all internal/external ratios (for both X/A and Y/B) obtained for the canine and the molar (palatal root and distobuccal root) were determined. Obtained results confirm a significant impact of the lower threshold value used for the development of the tooth model on relations between canal dimensions and external

*Table 2. Molar measurement results*

| | **Lower threshold value** | | | | | |
|---|---|---|---|---|---|---|
| | **400** | **800** | **1100** | **1300** | **1500** | **1700** |
| **Length of palatal root [mm]** | 23.20 | 23.20 | 23.04 | 22.88 | 22.88 | 22.72 |
| **Lengthof mesiobuccal root [mm]** | 21.28 | 21.28 | 21.12 | 20.96 | 20.80 | 20.72 |
| **Length of distobuccal root [mm]** | 20.64 | 20.64 | 20.48 | 20.32 | 20.00 | 19.84 |
| **Hard tissue volume [mm$^3$]** | 1502 | 1399 | 1323 | 1268 | 1203 | 1114 |
| **Internal/external dimensions ratio** | | | | | | |
| **Palatal root** | | | | | | |
| Level A | | | | | | |
| X/A | 0.250 | 0.269 | 0.360 | 0.360 | 0.375 | 0.435 |
| Y/B | 0.303 | 0.344 | 0.387 | 0.419 | 0.419 | 0.500 |
| Level B | | | | | | |
| X/A | 0.250 | 0.286 | 0.286 | 0.333 | 0.333 | 0.364 |
| Y/B | 0.350 | 0.410 | 0.421 | 0.432 | 0.486 | 0.486 |
| Level C | | | | | | |
| X/A | 0.231 | 0.320 | 0.348 | 0.348 | 0.435 | 0.476 |
| Y/B | 0.323 | 0.323 | 0.414 | 0.414 | 0.414 | 0.483 |
| **Mesiobuccal root** | | | | | | |
| Level A | | | | | | |
| X/A | 0.216 | 0.432 | 0.472 | 0.514 | 0.543 | 0.629 |
| Y/B | 0.125 | 0.304 | 0.318 | 0.381 | 0.429 | 0.450 |
| Level B | | | | | | |
| (Xa+Xb)/A | 0.204 | 0.306 | 0.396 | 0.489 | 0.596 | 0.696 |
| (Ya+Yb)/B | 0.250 | 0.286 | 0.444 | 0.500 | 0.538 | 0.520 |
| Level C | | | | | | |
| X/A | 0.476 | 0.512 | 0.561 | 0.590 | 0.615 | 0.667 |
| Y/B | 0.280 | 0.320 | 0.391 | 0.391 | 0.478 | 0.500 |
| **Distobuccal root** | | | | | | |
| Level A | | | | | | |
| X/A | 0.120 | 0.217 | 0.261 | 0.318 | 0.364 | 0.429 |
| Y/B | 0.176 | 0.200 | 0.333 | 0.333 | 0.462 | 0.538 |
| Level B | | | | | | |
| X/A | 0.314 | 0.314 | 0.353 | 0.353 | 0.424 | 0.485 |
| Y/B | 0.200 | 0.250 | 0.300 | 0.389 | 0.444 | 0.444 |
| Level C | | | | | | |
| X/A | 0.233 | 0.276 | 0.310 | 0.310 | 0.370 | 0.444 |
| Y/B | 0.222 | 0.222 | 0.353 | 0.353 | 0.437 | 0.500 |

root dimensions; correlation is increasing and nearly linear (in view of a too small sample size, no regression analysis was conducted). In addition, noteworthy is decreasing standard deviation value with increasing lower threshold value. In this case, significantly lower values for the threshold value ≥ 1100 HU (SD=0.038-0.043) as compared to SD=0.062 for the threshold value equal 400 HU were obtained.

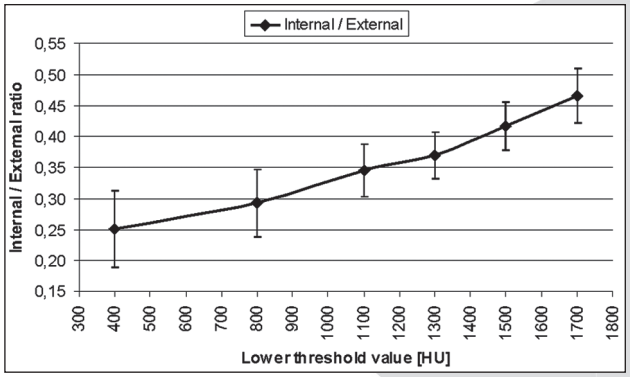


*Figure 6. Mean values and standard deviations of the internal/external ratio of examined teeth according to different threshold values.*

## Discussion

The usefulness of CBCT for the evaluation of root canal morphology was favorably assessed in clinical and *ex vivo* studies (8,10,11,18). Neelakantan et al. (11), on the basis of volume-rendering and multiplanar volume reconstructions, have found CBCT as accurate as modified canal staining and clearing technique and more precise than digital radiography and spiral CT in identifying the root canal anatomy. The probability of overlooking one or more root canals in these tests was 23.8% for digital radiographs, 15.58% for spiral CT, while for CBCT it was 0.29% only. Michetti et al. (10), by sliced analysis, evaluated the possibility of two-dimensional reconstructions of root canals based on cone beam computed tomography and they found it to be a reliable tool compared to histological data. However, Szabo et al. (12) found that some canals can be invisible in the apical part of the root and that the possibility of visualization of full canal depended on the CBCT equipment's parameters. In the literature, no reports on dental tissue reconstruction through the thresholding process based on volumetric tomography data can be found; only Hanning et al. (19) in the studies concerning flat-panel volumetric tomography used hard tissue segmentation based on the Hounsfield scale.

Hanning et al. (19) suggest that the volumes of the dental hard tissues may be underestimated depending on the different Hounsfield units defined for certain structures. Hara *et al.* (16) found that, even in case of μCT, a 0.5% change in the threshold resulted in a 5% difference in bone volume fraction. The present study revealed that the determination of thresholds plays an important role in the ability to three-dimensional visualization of external and internal dental anatomy. Provided 3D reconstructions revealed many anatomical details, e.g. canal splitting in the canine and extensive anastomosis between MB and MB2 canals, however, the quality of imaging of these details differed depending on the lower threshold value. Taking into consideration both outer and inner tooth structures, with increased lower threshold of the window we observed shortening of the tooth model's contours, while the pulp cavity was more and more extended and reconstructed in detail. The changes were confirmed also in quantitative terms. Differences in tooth length in the order of 0.5-0.8 mm are to be evaluated as clinically insignificant because in the course of endodontic treatment the working length of a canal is determined electronically, while correct imaging of the dental cavity structure with all details is of key importance for the endodontist. The differences we have shown in the root canal structure depending on the lower threshold value can influence both the attempt to search for further canals and the choice of the preparation and filling method. It seems that making 3D reconstructions of a tooth, realistically and precisely enough reflecting both outer and inner tooth structures, will in future require the use of more intelligent parameter selection algorithms of the thresholding process, taking into consideration different needs with regard to outer and inner tooth structures. Further research are required, e.g. the comparison of canal diameter at different levels with histological data. It should not be forgotten that our examination was conducted in *ex vivo* conditions, whereas in clinical conditions the tissue segmentation may cause further difficulties resulting from the presence of tissues surrounding the tooth in the CBCT image.

In general, determining optimal thresholding process parameters appears to be an essential ele-

ment of the three-dimensional tooth modeling. One of the most frequently used methods of bone or tooth segmentation from the image background is the global or local thresholding approach (20,21). According to Hassan at al. (17), the specification of correct threshold value of separate hard and soft tissues is more difficult in CBCT than in conventional CT. Hosntalab et al. (22) state that a single thresholding approach would result in missed dental structures (high threshold values) or cause adjacent bony tissues to attach to each other (low threshold values). When using local threshold value method, initial segmentation must be performed, which increases the number of factors influencing the quality of provided reconstructions (20). The other way is to choose a few different threshold values and compare the scans with the original one. However, in this case the threshold selection could be affected by a systemic error of a particular observer, and the process itself is a tedious work (16,23). Furthermore, other approaches are proposed to provide tooth segmentation, e.g. watershed or panoramic pre-sampling and variation level set (22).

Another aspect influencing the quality of 3D reconstructions based on CBCT data is the choice of such parameters as field of view (FoV) and voxel size (12,17,21). The disadvantages of full scanners, with FoV from 100 to 200 mm, are great beam angulation in outer parts of volume area and small contrast to noise ratio. The FoV of limited CBCT (dental or regional) may vary from 40 to 100 mm (23). A voxel size of CBCT devices varies from 50 to 200 μm for limited scanners to between 300 and 400 μm for full ones. For endodontic purpose, the first category is preferred because of higher spatial resolution improving the accuracy of the visualization of small features e.g. canal morphology, decrease of radiation exposure compared to medical CT and full CBCT scanners and shorter time of 3D reconstructions (12,24). In our study, FoV was 80x80 mm and voxel size was 160 μm. Voxel size is responsible for the partial volume effect. Maret et al. (5) found that, the accuracy of 3D CBCT reconstructions of the germs was high, but it depended on tooth thickness. In their study, the differences between μCT and CBCT data occurred mainly near the cervix of the germ and at the incisal edges of anterior teeth. The authors explained this phenomenon as a result of partial volume effect. Despite the fact that they used relatively small voxel size for CBCT data (76 μm) it was more than 85% higher than for μCT (41 μm). According to Michetti et al. (10), partial volume effect was responsible for the underestimation and smoothing of canal contours compared to histological sections. Szabo et al. (12) concluded that it was impossible to determine at what level the partial volume effect interfered with the visualization of the canal. The algorithms of segmentation applied in CBCT devices should limit the partial volume effect, but it still can affect the results and lead to overinterpretation of the generated three-dimensional reconstructions (19).

## Conclusions

Cone-beam computed tomography may be a useful tool for the evaluation of outer and inner dental structure in ex vivo studies. The possibility to make three-dimensional reconstructions of selected structures is a breakthrough in dental imaging. It must also be remembered that such modeling is extremely useful in the aspect of biomechanical tooth strength analysis and engineering evaluation of various endodontic techniques. However, the value of the lower threshold influences the quality of reconstruction of examined objects and the degree of visualization of details in the dental cavity structure. Therefore, the thresholding process should be carried out with special caution. A thesis may be proposed that until new, more intelligent algorithms for automatic selection of parameters of the thresholding process are developed, it will be necessary to provide „manual" evaluation of the influence of the use of another (lower and higher, respectively) value of the lower window threshold. That way the risk of making mistakes in the evaluation of CBCT examination results and wrong therapeutic decisions will be considerably reduced.

## References

1. *Lofthang-Hansen S, Huumonen S, Gröndahl K, Grönndahl HG. Limited cone-beam CT and intraoral radiography for the diagnosis of periapical pathology. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 103, 1: 114-119.*
2. *Nair MK, Nair UP. Digital and advanced imaging in endodontics: A Review. J Endod 2007; 33, 1: 1-6.*

3. *Nielsen RB, Alyassin AM, Peters DD, Carnes DL, Lancaster J. Microcomputed tomography: an advanced system for detailed endodontic research. J Endod 1995; 21,11: 561-568.*

4. *Bjørndal L, Carlsen O, Thuesen G, Darvann T, Kreiborg S. External and internal macromorphology in 3D-reconstructed maxillary molars using computerized X-ray microtomography Int Endod J 1999; 32, 1: 3-9.*

5. *Maret D, Molinier F, Braga J, Peters OA, Telmon N, Treil J, Inglèse JM, Cossié A, Kahn JL, Sixou M. Accuracy of 3D reconstructions based on cone beam computed tomography. J Dent Res 2010; 89, 12: 1465-1469.*

6. *Uzbek U, Rahman SA, Hindi RM, Gillami SW, Athar Y. Life in 3D: A 10 year review of literature on the application of cone beam computed tomography in dental implantology. HealthMED 2012; 6, 5: 1754-1762.*

7. *Gao Y, Peters OA, Wu H, Zhou X. An application framework of three-dimensional reconstruction and measurement for endodontic research. J Endod 2009; 35, 2: 269-274.*

8. *Matherne RP, Angelopoulos C, Kulild JC, Tira D. Use of cone-beam computed tomography to identify root canal system in vitro. J Endod 2008; 34, 1: 87-89.*

9. *Liang X, Lambrichts I, Denis K, Hassan B, Li L, Pauwels R, Jacobs R. A comparative evaluation of cone beam computed tomography (CBCT) and multislice CT (MSCT). Part II: On 3D model accuracy. Eur J Radiol 2010; 75, 2: 270-274.*

10. *Michetti J, Maret D, Mallet JP, Diemer F. Validation of cone beam computed tomography as a tool to explore root canal anatomy. J Endod 2010; 36, 7: 1187-1190.*

11. *Neelakantan P, Subbarao C, Subbarao CV. Comparative evaluation of modified canal staining and clearing technique, cone-beam computed tomography, peripheral quantitative computed tomography, spiral computed tomography, and plain and contrast medium-enhanced digital radiography in studying root canal morphology. J Endod 2010; 36, 9: 1547-1551.*

12. *Szabo BT, Pataky L, Mikusi R, Fejerdy P, Dobo-Nagy C. Comparative evaluation of cone-beam CT equipment with micro-CT in the visualization of root canal system. Ann Ist Super Sanita 2012; 48, 1: 49-52.*

13. *Piszczatowski S, Baginska J, Swieszkowski W. Modelling of tooth's structure based on CT and μCT data – comparative study. IFMBE Proceedings 2008; 22: 1463-1466.*

14. *Buzug TM. Practical aspects of computed tomography, [in:] Computed tomography: from photon statistic to modern cone-beam CT, Springer, Berlin, Heidelberg. 2008: 471-484.*

15. *Ding M., Odgaard A., Hvid I., Accuracy of cancellous bone volume fraction measured by micro-CT scanning, Journal of Biomechanics, 1999, 32, 3, 323-326*

16. *Hara T, Tanch E, Homminga J, Huiskes R. The influence of microcomputed tomography threshold variations on the assessment of structural and mechanical trabecular bone properties. Bone 2002; 31, 1: 107-109.*

17. *Hassan B, Souza PC, Jacobs R, de Azambuja Berti S, van der Stelt P. Influence of scanning and reconstruction parameters on quality of three-dimensional surface models of the dental arches from cone beam computed tomograph., Clin Oral Invest 2010; 14, 3: 303-310.*

18. *Zhang R, Wang H, Tian Y.-Y, Yu X, Hu T, Dummer PMH. Use of cone-beam computed tomography to evaluate root and root canal morphology of mandibular molars in Chinese individuals. Int Endod J 2011; 44, 11: 990-999.*

19. *Hanning C, Krieger E, Dullin C, Merten HA, Attin T, Grabbe E, Heidrich G. Volumetry of human molars with flat panel-based volume CT in vitro. Clin Oral Invest 2006; 10, 3: 253-257.*

20. *Loubele M, Maes F, Schutyser F, Marchal G, Jacobs R, Suetens P. Assessment of bone segmentation quality of cone-beam CT versus multislice spiral CT: a pilot study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006; 102, 2: 225-234.*

21. *Al-Rawi B, Hassan B, Vandenberge B, Jacobs R. Accuracy assessment of three-dimensional surface reconstructions of teeth from cone beam computed tomography scans. J Oral Rehab 2010; 37, 5: 352-358.*

22. *Hosntalab M, Zoroofi RA, Tehrani-Fard AA, Shirani G. Segmentation of teeth in CT volumetric dataset by panoramic projection and variation level set. Int J Comput Assist Radiol Surg 2008; 3, 3-4: 257-265.*

23. *Patel S, Dawood A, Pitt Ford T, Whaites E. The potential applications of cone beam computed tomography of endodontic problem., Int Endod J 2007; 40, 10: 818-820.*

24. *De Vos W, Casselman J, Swennen GRJ. Cone-beam computerized tomography (CBCT) imaging of the oral and maxillofacial region: A systematic review of the literature. Int J Oral Maxillofac Surg 2009; 38, 6: 609-625.*

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# Knowledge, attitude and behaviour of medical and health school students on breast cancer and breast self-examination

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**Abstract**

**Objective:** We aimed to determine the knowledge, attitudes, and behaviour of female medical and health school students in relation to breast cancer and breast self-examination (BSE).

**Methods:** This study was conducted in a medical and health school in Turkey. A total of 288 (82.3%) of 350 female students were reached in the school of health, and 49 (73.1%) of 67 female students were reached in the medical school. The data were collected by a standard questionnaire comprising 32 questions.

**Results:** A total of 55.8% of the students evaluated their knowledge of BSE as inadequate. A total of 78.0% of the students had received no training about BSE, and 72.1% of the students believed that breast cancer was the most common type of cancer among women. In terms of performing BSE, 70.9% of the participants performed the procedure, 64% on a monthly basis and 5% during menstruation.

**Conclusions:** These medical and health school students receive inadequate education on BSE and breast cancer and fail to perform BSE in a regular and timely fashion. They should be instructed thoroughly on this topic prior to graduation, and they should learn to practice BSE on a regular and timely basis.

**Key words:** Breast self-examination, breast cancer, student, Turkey.

## Background

Breast cancer is an important health problem across the world and is the most common cancer type among women. It is one of the leading causes of death in several countries. It is the second leading cause of death in the U.S. (1) and the third in Korea (2). In Turkey, breast cancer is the most common type of cancer among women and comprises 24.1% of all cancer cases (3).

The diameter of the tumour and involvement of axillary lymph nodes are the most important factors influencing the prognosis in breast cancer. Increased tumour diameter and the involvement of the axillary lymph nodes raise mortality and local recurrence rates (4). Therefore, early diagnosis in breast cancer facilitates treatment and prolongs the life span of the patient (5). Mammography, clinical breast examination, and breast self-examination (BSE) are recommended in the early diagnosis of breast cancer (6). When performed properly and at regular intervals, BSE is a simple and cost-effective method, which also preserves the privacy of female patients (5). Women (81%) are generally the first to recognise changes in their breasts (5). In Finland, 28,785 of women who had been taught to perform BSE were compared with those who had not received such training (7). Although no difference was determined in terms of cancer stage, the mortality rate was decreased (7). Based on findings from the Kotka pilot project in Finland, the authors noted that routine BSE is important with regard to early diagnosis of breast cancer, while also reducing mortality (8).

According to previous studies in Turkey, the general Turkish population does not have adequate knowledge of breast cancer, and many women either do not know how to perform BSE or simply do not perform it (9). Karayurt, Coşkun, and Cerit found that only 32% of nurses regularly performed BSE (11). Although there are many studies on BSE involving midwives and nurses,

the number of studies on female students receiving medical education, particularly those studying in medical schools, are limited.

In this study, we aimed to shed a light on future training activities by determining the knowledge, attitudes, and behaviour of female medical and health school students in relation to breast cancer and BSE.

## Methods

The city of Kahramanmaras has a population of 430, 000 people and is located in the Eastern-Mediterranean region of Turkey. All our students are Muslims and originate from families of average socio-economic level.

This cross-sectional study was conducted among female medical school and health school (nursing and midwifery) students of the Kahramanmaras Sutcuimam University between November 2010 and February 2011. A member of the study team, a nurse-midwife instructor, asked the students to complete a questionnaire during an ordinary class and explained that participation was voluntary. The forms were completed in the classroom, left in a closed box, and collected by the project leader. The confidentiality of the respondents was assured.

The questionnaires included nine questions aimed at determining the socio-demographic characteristics and 25 questions on breast cancer and BSE. The research protocol was reviewed and approved by the Institutional Review Boards" of the medical center and the county board of education.

The questionnaire had been tested previously in a pilot project involving 20 female technical vocational school students studying health.

All variables were expressed as numbers and percentages. The Chi-square test was used for the statistical analysis; $p$ values <0.05 were considered statistically significant. All the data were entered into and processed by Epi Info 2002 software (CDC, Atlanta, GA, USA).

## Results

In total, 337 (80.1%) of 417 female undergraduate students in the above-mentioned schools were reached. Forty-nine (14.5%) of the students were from the medical school, and 288 (85.5%) (161 midwifery and 127 nursing students) were from the school of health. The mean age of the participants was 20.38 ± 1.82 years. Sixteen (4.7%) of the students were smokers.

### *Students' knowledge of breast cancer and breast self-examination*

Two hundred forty-three of the participants (72.1%) knew that breast cancer was the most common type of cancer. Three hundred-fourteen of the participants (93.2%) said that "The risk of breast cancer development would be higher if the mother or sister had breast cancer." The students said that the most common signs of breast cancer were as follows: a palpable breast lump, breast pain, and a palpable axillary lump. When asked for measures that would allow early diagnosis of breast cancer, 95.0% responded with BSE, whereas 81.3% and 76.0% said that mammography and clinical examination, respectively, would help. A total of 41.8% of the students did not know whether breast cancer could be encountered in males. A total of 94.4% of the students thought that smoking elevated the risk of breast cancer. Three hundred-six students (90.8%) knew that all adult females should perform BSE. Only twenty-seven of the students (8%) answered the question "When is the proper time to perform BSE?" correctly as "During menstruation".

Senior students had statistically a significantly higher level of knowledge of BSE ($p$ <0.001).

Details on the knowledge of the medical school and the health school students on breast cancer and BSE are shown in Table 1.

Attitudes of the medical school and the health school students towards breast cancer and breast self-examination

A total of 55.8% of the students evaluated their knowledge of BSE as inadequate. When only final-year students were considered, this rate dropped to 32.4%. Twenty-two percent of the students had received a course, lesson, or in-service training about BSE. A total of 58.5% had gained their knowledge of BSE from their current school, and the BSE rate was statistically significantly higher among the students with a higher knowledge of BSE ($p$ <0.001).

Among the students who had not performed BSE, 124 (36.7%) said that "I found BSE unnecessary in the absence of a complaint." As shown in

*Table 1. Knowledge of medical school and health school students on breast cancer and breast self-examination*

| Item | n | (%) |
|---|---|---|
| Breast cancer was the most common cancer type. | 243 | 72.1 |
| The risk of breast cancer development would be higher if the mother or sister had breast cancer. | 314 | 93.2 |
| All adult females should perform BSE. | 306 | 90.8 |
| BSE would allow early diagnosis of breast cancer. | 320 | 95.0 |
| Clinical examination would help early diagnosis of breast cancer. | 256 | 76.0 |
| Mammography would allow early diagnosis of breast cancer. | 274 | 81.3 |
| I do not know whether males can get breast cancer. | 141 | 41.8 |
| Smoking elevates the risk of breast cancer. | 318 | 94.4 |
| ***What are the signs of breast cancer?*** | | |
| May be no sign at all | 69 | 20.5 |
| Breast pain | 269 | 79.8 |
| Palpable breast lump | 306 | 90.8 |
| Palpable axillary lump | 264 | 78.3 |
| Inverted nipple | 139 | 41.2 |
| Bloody discharge from nipple | 154 | 45.7 |
| Yellow discharge from nipple | 111 | 32.9 |
| Unilateral breast deformity | 196 | 58.2 |
| Wrinkled breast skin | 88 | 26.1 |
| Feeling of tightness in breast | 100 | 29.7 |
| Breast wound | 83 | 24.6 |
| Sensation of burning in breast | 92 | 27.3 |
| ***When should you start BSE?*** | | |
| >20 years old (correct answer) | 34 | 10.1 |
| Wrong answer or no answer | 303 | 89.9 |
| ***In which age group is breast cancer more common?*** | | |
| >40 years of age (correct answer) | 129 | 38.3 |
| Wrong answer or no answer | 208 | 61.7 |
| ***What is the correct time to perform BSE?*** | | |
| During menstruation (correct answer) | 27 | 8.0 |
| Wrong answer or no answer | 310 | 92.0 |

*Table 2. Attitudes of medical school and health school students towards breast cancer and breast self-examination*

| Item | n | (%) |
|---|---|---|
| I have inadequate knowledge of BSE. | 188 | 55.8 |
| I received a course, lesson, or in-service training about BSE. | 74 | 22.0 |
| I gained my knowledge of BSE from my current school. | 197 | 58.5 |
| I do not know how to perform the procedure. | 172 | 51.0 |
| I found BSE unnecessary in the absence of a complaint. | 124 | 36.7 |
| BSE is unnecessary due to the absence of breast cancer in my family. | 34 | 10.1 |
| I did not realise the importance of BSE. | 7 | 2.0 |

*Table 3. Behaviour of medical school and health school students in relation to breast cancer and breast self-examination*

| Item | n | (%) |
|---|---|---|
| ***The question "Do you perform BSE?" answered "Yes"*** | 239 | 70.9 |
| Medical school (*n* = 49) | 23 | 46.9 |
| School of health (*n* = 288) | 216 | 75.0* |
| midwifery students (*n* = 161) | 132 | 82.0* |
| nursing students (*n* = 127) | 84 | 66.1* |
| ***Time when students who carried out BSE performed the procedure (n = 239)*** | | |
| During menstruation (correct answer) | 12 | 5.0 |
| Wrong answer or no answer | 227 | 95.0 |
| ***Number of times these students performed BSE (n = 239)*** | | |
| Once a month (correct answer) | 153 | 64.0 |
| Wrong answer or no answer | 86 | 36.0 |
| ***Number of students who had visited a physician for breast examination*** | 16 | 4.7 |

**Chi-Square Test, p <0.05 statistically significant.*

Table 2, the medical school students and the health school students exhibited a similar attitude towards breast cancer and BSE.

***Behaviour of the medical school and the health school students in relation to breast cancer and breast self-examination***

A total of 70.9% of the students reported that they performed BSE, whereas 29.1% noted that they did not. The BSE rate among the medical school students was 46.9%, whereas it was 75.0% among the health school students. The difference was statistically significant (p <0.001). A total of 82.0% of the midwifery students stated that they performed BSE, whereas the rate was 66.1% among the nursing students. The difference was statistically significant (p <0.001). One hundred seventy-two students (51.0%) did not know how to perform BSE, and 124 (36.7%) of the students stated that they found it unnecessary in the absence of a complaint. Table 3 provides information on the behaviour of the medical school and the health school students in relation to breast cancer and BSE.

## Discussion

It is important that students in health-related universities should gain adequate knowledge of certain medical conditions requiring early detection and prevention and that they perform these themselves before graduation. For female students, BSE is one such medical condition.

More than half (55.8%) of the students evaluated their own knowledge level of BSE as inadequate. When only final-year students were considered, the knowledge level (32.4%) was still inadequate. Only 22.0% had received a course, lesson, or in-service training on BSE. A total of 58.5% had gained their knowledge of BSE from their current schools. Similar results were reported by Uzun, Karabulut, and Karaman in a study of nursing students and by Aydın in a study of health school students (12,13). Preventive healthcare methods should be taught during initial university years, and the students should develop the ability to practice these procedures in their own lives.

Eleven percent of the students had a relative, such as a mother or a sister, who had breast cancer. In a study conducted on midwifery students, a rate of 7.7% was reported (14). The majority (93.2%) of the students thought that if their mothers or any of their sisters had breast cancer, they would have a greater risk of developing breast cancer. Individuals with a family member suffering from breast cancer are inclined to feel that they have a greater risk of the disease (15). The presence of a family member with breast cancer motivates people to be more sensitive towards the disease and prompts them to gain knowledge about it, while perceiving the outcomes of the disease as life threatening.

The most frequently cited symptoms of breast cancer were a palpable breast lump, breast pain, and an axillary palpable lump (Table 1). In the study of Aslan et al., nursing school students

thought that the most common symptoms of breast cancer were a palpable mass and pain (16).

In response to the question "What methods could help early detection of breast cancer?", 95.0% answered BSE, 81.3% answered mammography, and 76.0% answered a clinical breast examination. Monthly BSE, regular mammography, and occasional clinical breast examination are recommended for early detection of breast cancer (17,18).

When the students were asked about the age for starting BSE, 43.0% stated that it should commence before the age of 18 years, 46.9% believed that it should be 18–20 years, and 10.1% thought that it should be later than age 20 years. In the study of Uzun et al., 96% of the nursing students stated that BSE should commence during adolescence (12). The World Health Organization, the Cancer Control Program, and the National Cancer Institute recommend starting BSE at the age of 20 years for early detection of breast cancer (19). The results of both our study and those of others show that students in health-related universities do not know the correct age to start performing BSE.

Most of the students (90.8%) stated that all adult women should perform BSE, and 70.9% of the students said that they performed BSE. The BSE rate was 46.9% among the medical school students and 75.0% among the health school students. The difference was statistically significant ($p<0.001$). Moreover, the BSE rate was 82.0% among the midwifery students and 66.1% among the nursing students. The difference was again statistically significant ($p<0.001$). A total of 51.0% of the students who had not performed BSE stated that they did not know how to perform the procedure (Table 2). Aslan et al. found the BSE rate among nursing students was 63% (16). A BSE rate of between 5.5 and 32% has been reported among Turkish women (9,20). Those results indicate low BSE rates, both among female students in health-related universities and in the general population. The current number of studies on BSE practices among students in medical schools is not adequate. The curriculum of health-related universities should be revised to teach students how to perform BSE, and they should be encouraged to perform BSE in their own lives.

According to 8.0% of the students, the best time to perform a BSE was during menstruation, and 5.0% of those who performed BSE did so during menstruation. A total of 13.0% of the medical students performed BSE after menstruation, and 8.6% of the health school students did so during menstruation. The difference was statistically significant ($p <0.001$). In another study, 68.8% of nursing students performed BSE at the correct time (12). In our study, the percentage of the medical school students who performed BSE at the correct time was 13.0%, which is very low. Among the medical school students, the BSE rate was also low, and the timing of the BSE was incorrect (Table 3).

In our study, 64.0% of the students who performed BSE did so on a monthly basis. Budden found that 27.0% of nursing students performed BSE regularly; Aslan reported a rate of 41.3% (16,21).

In the early detection of breast cancer, clinical examination and mammography are considered to be the first steps towards diagnosis (22,23). In the current study, 4.7% of the students had underwent a clinical breast examination, and 1.5% had underwent mammography.

In our study, 4.7% of the participants were smokers. Smoking is a known serious risk factor for breast cancer (24).

This study had some limitations. First, our study was designed as a cross-sectional survey and did not include monitoring of the participants. Data were collected by self-report. The participants might have made mistakes because the performance frequency of BSE is a measurement based on remembering. The health beliefs and BSE practices of individuals also change over time. Second, in this study, the sample size was relatively small. As it involved only female students from the school of health and medicine of KSU, Turkey, these results cannot be generalised to female Turkish students attending other schools of health and medicine. Further studies are needed using larger samples in different universities in Turkey.

In conclusion, the study showed that these medical school and health school students receive inadequate education on BSE and that they perform BSE irregularly at improper times. Prior to graduation, their lack of knowledge on this subject should be resolved, and they should be taught how to perform BSE properly at correct times and regular intervals.

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**References**

1. *Tittle, M., Chiarelli, M., McGough, K., McGee, S. J., & McMillan, S. (2002). Women's health beliefs about breast cancer and health locus of control. Journal of Gerontological Nursing, 28, 37-45.*

2. *Lee, C. Y., Kim, H. S., & Ham, O. (2000). Knowledge, practice, and risk of breast cancer among rural women in Korea. Nursing and Health Sciences, 2, 225-230. doi: 10.1046/j.1442-2018.2000.00063.x*

3. *Republic of Turkey Ministry of Health, Cancer Control Policy and the Cancer Data 1995-1999 [T.C Sağlık Bakanlığı Kanserle Savaş Politikası ve Kanser Verileri 1995-1999] (2002). Kanser Savaş Dairesi Başkanlığı Yayını, Yayın No:168, Ankara*

4. *Rimer, B. K. (1996). Breast cancer screening. In J. R. Harris, M. E. Lippman, M. Morrow, & S. Hellman (Eds), Diseases of the breast. Philadelphia, USA: Lippincott Co.*

5. *Dozier, K. J., & Mahon, S. M. (2002). Cancer prevention, detection and control: A nursing perspective (pp. 389-443). Oncology Nursing Society. Pittsburg Press.*

6. *American Cancer Society (ACS). Cancer facts and figures 2006. Atlanta: American Cancer Society; 2006. Retrieved from http://www.cancer.org/acs/groups/content/@nho/documents/document/caff2006pwsecuredpdf.pdf*

7. *Gastrin, G., Miller, A. B., To, T., Aronson, K. J., Wall, C., Hakama, M., Louivouri, K., & Pukkala, E. (1994). Incidence and mortality from breast cancer in the mama program for screening in Filland, 1973-1986. Cancer, 73, 2168-2174.*

8. *Hakama, M., Pukkal, E., Kallio, M., Godenhjelm, K., & Svinhufvud, U. (1995). Effectiveness of screening for breast cancer in women under 50 years at entry: the Kotka pilot project in Finland. International Journal of Cancer, 63, 55-57. doi: 10.1002/ijc.2910630111*

9. *Secginli, S., & Nahcivan, N. O. (2004). Reliability and validity of the breast cancer screening belief scale among Turkish women. Cancer Nursing, 27, 287-294.*

10. *Çadır, G., Eksen, M., Bütüner, E., Tüzen, H., Yetim, H., Othan, K., Arslan, K. (2004). Determining knowledge level and application self breast check (SBC) and breast cancer of women in Muğla county, Bayır, Yerkesik and Yeşilyurt health center areas. International Journal of Human Science, 1, 1-12. Retrieved from http://www.insanbilimleri.com/ojs/index.php/uib/article/view/124*

11. *Karayurt, O., Coşkun, A., & Cerit, K. (2008). Nurses' beliefs about breast cancer and breast self examination and their breast self examination performance. Journal of Breast Health, 4, 15-20.*

12. *Uzun, O., Karabulut, N., & Karaman, Z. (2004). Knowledge and Practices of Nursing Students about Breast Self-Examination. Ataturk University Nursing Collage Journal, 7, 33-41.*

13. *Aydın, I. (2004). Knowledge and practices about breast self examination of university students. Ataturk University School of Nursing Journal, 7, 26-34.*

14. *Beydağ, K. D., & Yürügen, B. (2010). The effect of breast self-examination education given to midwifery students on their knowledge and attitudes. Asian Pacific Journal of Cancer Prevention, 11, 1761-1764.*

15. *Powe, B. D., Underwood, S., Canales, M., & Finnie, R. (2005). Perceptions about breast cancer among college students: Implications for nursing education. Journal of Nursing Education, 44, 257-265.*

16. *Aslan, A., Temiz, M., Yiğit, Y., Can, R., Canbolat, E., & Yiğit, F. (2007). The knowledge attitude and behaviorus of nursery students about breast cancer. TAF Preventive Medicine Bulletin, 6, 193-198. Retrieved from http://www.ejmanager.com/mnstemps/1/khb_006_03-193.pdf?t=1353423608*

17. *Gross, R. E. (2001). Assessment and management of patients with breast diseases. In S., C., Smeltzer, & B., G., Bare (Eds). Brunner and Suddarth's Textbook of Medical–Surgical Nursing (9th edition), (pp.1259-1296). Philadelphia, New York, Baltimore: Lippincott Williams Wilkins Co.*

18. *Sadler, G. R., Dhanjal, S. K., Shah, N. B., Shah, R. B., Ko, C., Anghel, M., & Harshburger, R. (2001). Asian Indian Women: Knowledge, attitudes and behaviors toward breast cancer early detection. Public Health Nursing, 18, 357-375. doi: 10.1046/j.1525-1446.2001.00357.x*

19. *Gerald, D., & Dodd, M. (1992). American cancer society guidelines on screening for breast cancer. Cancer Supplement , 69, 143-145.*

20. *Secginli, S., & Nahcivan, N. O. (2006). Factors associated with breast cancer screening behaviors in a sample of Turkish women: a questionnaire survey. International Journal of Nursing Studies, 43, 161-171.*

21. *Budden, L. (1995). Young women's breast self-examination knowledge and practice. Journal of Community Health Nursing,12, 23-32.*

22. *Sorensen, J., & Hertz, A. (2003). Cost-effectiveness of a Systematic Training Program in Breast Self-examination. European Journal of Cancer Prevention, 12, 289-294.*

23. *Crossing, S., & Manaszewicz, R. (2003). Breast self-examination: Be alert but not alarmed. Medical Journal of Australia, 178, 646-647.*

24. *Özkahraman, S., Vural, B. K., & Bayık, A. (2006). Improving the breast self examination skills of women attenting public education center. Ataturk University Nursing Collage Journal, 9, 1-9.*

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# Serum prolidase activity in patients with carpal tunnel syndrome

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**Abstract**

**Introduction:** Up until now, there is no study in the medical literature that evaluates serum prolidase activity in patients with carpal tunnel syndrome (CTS). Our aim in this study was to determine serum activity of prolidase in CTS patients versus controls to determine whether its activity reflects disturbances of collagen metabolism. We also investigated the relationship of serum prolidase activity and electrophsiological parameters in CTS patients.

**Methods:** Forty-five patients with CTS were prospectively enrolled in the study. The CTS diagnosis was confirmed with the clinical, electrophysiological examination and the Boston survey. The control group comprised 46 healthy volunteers. Serum prolidase activities (U/L) were measured by spectrophotometric method.

**Results:** There was no difference between CTS patients and controls in age and gender ($p>0.05$). Serum prolidase activity (707.4±217.6 U/L) in patients with CTS were statitistically significantly higher compared with control (622.6±148.1 U/L) ($p=0.03$). A positive correlation was found between the prolidase activity and median sensory distal latency (mSDL) ($p=0.029$, $r=0.34$) and difference between median and ulnar sensory latencies from digit 4 stimulation (D4M-D4U) ($p=0.039$, $r=0.32$) in CTS patients. Also, a negative correlation was observed between the serum prolidase activity and median sensory nerve conduction velocity (mSNCV) ($p=0.009$, $r=-0.40$) in CTS patients.

As a result, the increased serum prolidase activity in patients with CTS revealed in this study may suggests the presence of increased collagen turnover in CTS, which might play a key role in the progression of synovial fibrosis.

**Key words:** Prolidase, carpal tunnel syndrome, fibrosis.

## Introduction

Carpal tunnel syndrome (CTS) is the most common peripheral nerve entrapment. The entrapment occurs due to the fact that the compression of the median nerve hereby of the increased intra-canal compression as the nerve, together with the flexor tendons, passes through the carpal tunnel in the wrist beneath the the transverse carpal ligament (1). The prevalence of CTS in the general population is nearly 5% (2). Its etiology is not totally known. Fibrosis of the transverse carpal ligament (TCL), contribute to the symptoms (3). CTS is characterized clinically by pain, pins and needles in the hands. Conditions that have recurrently been associated with an increased prevalence in CTS patients are diabetes mellitus, thyroid Diseases, rheumatoid arthritis, gestation, and obesity (4). Fibrosis of the subsynovial connective tissue is the most characteristic histopathologic finding in patients with CTS. (5) Collagen types I and III were disrupted within the subsynovial connective tissue in patients with CTS (6). The histopathological features of the flexor tendon sheath in idiopathic CTS typically reveal a non-inflammatory fibrous connective tissue, with thickening of the tendon sheath, fibrosis, thickening of vascular walls. These findings support the view that compression in the carpal tunnel and ischemia are critical factors in the etiology of idiopathic CTS (7). Collagen is a protein, that is the major component of the connective tissue (8). Prolidase is a member of the matrixins family. It plays a major role in collagen turnover (9). The last stage of collagen degradation is mediated by prolidase, which is a cytosolic exopeptidase that cleaves iminodipeptides

containing carboxyterminal proline or hydroxyproline, and plays a significant role in collagen metabolism (10). These dipeptides are produced upon degradation of the collagen (8). However, to the best of our knowledge, there is no study in the medical literature that evaluates prolidase activity in the serum of patients with CTS. Our aim in this study was to determine serum levels of prolidase in CTS patients against controls to determine whether its activity reflects disturbances of collagen metabolism. We also investigated the relationship of serum prolidase and electrophysiological parameters in CTS patients. This study was designed to investigate the hypothesis that the serum prolidase activity could contribute to the development of the CTS.

## Material and Methods

Forty-five patients (38 female and 7 male) with CTS who applied to Dicle University Hospital Neurology clinic and electromyography (EMG) laboratory were prospectively enrolled in the study. Patients with polyneuropathy, trauma, systemic or metabolic diseases which may influence the nerve conductions (infection, diabetes mellitus, hypothyroidism, rheumatoid arthritis, amyloidosis, pregnancy and chronic renal failure undergoing hemodialysis) were excluded. The control group comprised 46 healthy volunteers. No additional interventional procedure or additional non-routine procedures were performed either on the patients with CTS group. The patients underwent detailed neurological examinations. The CTS diagnosis was confirmed with the clinical examination and the Boston survey. The Boston survey was performed in order to assess the functional symptom severity and functional status of the patients with CTS. The Boston survey consists of two components: Symptom severity scale (SSS) with 11 items and functional status scale (FSS) with 8 items(11) The clinical diagnosis of CTS was made based on the presence of at least two of the following conditions:

1) Pain and numbness in the affected hand, associated with activity and arising at night;
2) Paresthesia occurring in relation with manoeuvres that cause the median nerve to stretch (Phalen's test, Tinel's sign);
3) Weakness and atrophy in muscles innervated by the median nerve,
4) Median sensorial deficit.

CTS group underwent median and ulnar nerve sensorimotor nerve conduction study (NCS). The NCS were performed on standard electrodiagnostic equipment (Nihon Kohden, Japan). The median and ulnar nerves were stimulated at the wrist and elbow (antecubital space for median nerve, ulnar fossa for ulnar nerve) at a distance of 8 cm from the wrist to the active electrode. Sensory responses were obtained antidromically. Ring electrodes were used to obtain sensory nerve action potentials. Electrodes were placed over the second finger for the median nerve and fifth finger for the ulnar nerve. Also, the difference between median and ulnar latencies from D4 stimulation (D4M-D4U) were calculated in each subject. Motor conduction studies were performed from the median (recorded at the musculus abductor pollicis brevis), ulnar (recorded at the abductor digiti quinti). (1, 11). Patients with median motor and sensory nerve conduction velocities ≤50 m/s were defined as the CTS group (1).

### *Blood sampling*

After an overnight fasting, 10 cc venous blood was withdrawn into heparinized tubes. Remaining blood was centrifuged at 3500 × g for 5 minutes to separate the plasma and the serum specimens were stored at − 20°C until analysis.

### *Measurement of prolidase*

Prolidase activity (U/L) was determined by a spectrophotometric method which is determined by proline levels produced by prolidase. The supernatant was diluted twofold with physiologic saline (0.09% NaCl). Twenty-five microliters of the mixture were preincubated with 75 μL of the preincubation solution (50 mmol/L Tris HCl buffer pH 7.0 containing 1 mmol/L glutathione and 50 mmol/L MnCl 2 ) at 37°C for 30 minute. The reaction mixture, which contained 144 mmol/L gly–pro, pH 7.8 (100 μL), was incubated with 100 μL of preincubated specimen at 37°C for 5 minute. To stop the incubation reaction, 1 mL glacial ethanoic acid was added. After adding 300 μL Tris HCl buffer, pH 7.8 and 1 mL triketohydrindene hydrate solution (3 g/dL triketohydrindene hydrate was melted in 0.5 mol/L orthophosphoric acid), the mixture was incubated at 90°C for 20 minute and then cooled with

ice. Absorbance was then measured at a 515-nanometer wavelength bandwidth to determine proline by the method proposed by Myara et al.(12). This method is a modification of Chinard's method (13).

***Statistical method***

Mean values and standard deviations of the electrophysiological data for CTS group was evaluated using the Statistics Package for Social Sciences for Windows, SPSS version 11.5. The mean prolidase activities of the control and CTS groups were compared by the independent Student's t-test. The Chi-square test was used for the comparison of the nominal data. Relationships between variables were analyzed by Pearson correlation analysis. A $p<0.05$ was considered to be statistically significant.

## Results

This study included 38 female and 7 male CTS patients. Healthy subjects comprised 41 female and 5 male ($p>0.05$). The mean age of the CTS group was 37.2 ± 7.6 years and control group was 37.9 ± 5.6 years ( $p> 0.05$). Prolidase activity (707.4±217.6 U/L) in patients with CTS were statitistically significantly higher compared with control (622.6±148.1 U/L) ($p=0.03$). The electrophysiological results, FSS and SSS of the CTS group are shown in Table 1. A positive correlation was found between the prolidase activity and median sensory distal latency ($p=0.029$, $r=0.34$) and D4M-D4U ($p=0.039$, $r=0.32$) in CTS patients. Also, a negative correlation was observed between the prolidase activity and median sensory nerve conduction velocity (mSNCV) ($p=0.009$, $r=-0.40$) in CTS patients. No statistically significantly correlations were detected between prolidase activity, SSS, and FSS values in CTS patients ($P > 0.05$). Table 1

*Table 1. The electrophysiological results, FSS and SSS of the CTS group*

| | |
|---|---|
| FSS | 21.3±3.0 |
| SSS | 32.3±3.6 |
| Median sensory velocity (ms) | 34.3±5.6 |
| Median motor velocity (ms) | 57.6±3.7 |
| Median sensory distal latency (ms) | 3.2±0.6 |
| Median motor distal latency (ms) | 4.7±0.7 |
| median-ulnar distal latency difference (ms) | 1.3±0.8 |

*ms: millisecond, FSS: functional status scale, SSS: symptom severity scale, CTS: carpal tunnel syndrome*

## Discussion

In present study, we found that serum prolidase activity were significantly higher in patients

with CTS than controls. In patients with CTS, prolidase activity was positively correlated with mSDL as well as with M4U4, and prolidase activity was negatively correlated with mSNCV.

The increased prolidase activity in patients with CTS revealed in this study may suggests the presence of increased collagen turnover in CTS, which might play a key role in the progression of synovial fibrosis. The synovial tissues in the carpal tunnel are unique in containing a substantial, multilayered subsynovial connective tissue. Normally, the subsynovial connective tissue loosely connects the finger flexor tendons and median nerve to the visceral synovial membrane, which in turn encloses the tendons and nerve within the ulnar tenosynovial bursa. This sliding system is grossly disrupted in patients with idiopathic CTS (5, 14, 15). The most common histological finding in CTS is noninflammatory synovial fibrosis. The accumulated effect of minor injuries is believed to be an important etiologic factor in some cases of CTS. It has been suggested that consistently damage on subsynovial connective tissue can result connective tissue damage in patient with CTS (14). Recent studies suggest that in patients with CTS, pathological changes occur in the subsynovial connective tissue. Such changes are non-inflammatory synovial fibrosis and vascular proliferation. Thickening of the tendon sheet may cause an increase of canal pressure and damages to the median nerve in the wrist; however, the causes of such events still remain to be clarified (14, 16). Oh et al. noted some differences in ultrastructurally collagen morphology in patients with CTS, contrasted with individuals with no history of CTS (17). It has been shown that it is possible to measure subsynovial connective tissue thickness with sonography, and the tissue is thicker in patients with carpal tunnel syndrome than in healthy controls (18). Also, it has been found that increased vascularization of flexor tenosynovium is associated with patients' functional status (19). Kim et al., suggested that oxidative stress in subsynovial connective tissue is associated to CTS and its symptoms (20). Collagen biosynthesis may

require prolidase activity to play an important role in the break down of collagen and intracellular protein, specially in the end-stage when peptides and dipeptides contain a high level of proline (21). Collagen is essential for the maintenance of connective tissue. It has been shown that prolidase activity in normal fibroblasts is regulated by the interaction of extracellular matrix proteins, mainly type I collagen (22). Prolidase is a widely distributed cytosolic enzyme involved in the degradation of the collagen structure. The enzyme hydrolyzes imidodipeptides and imidotripeptides with C-terminal proline or hydroxyproline on the helical structure of collagen and releases the two amino acids for collagen resynthesis and cell growth (8). These imidopeptides originate from the intracellular degradation of procollagen, an extracellular form of collagen and the degradation of other proline and hydroxyproline containing proteins and dietary proteins. In addition to other collagenases, prolidase has a role in the end-stage of the collagen breakdown, and the enzyme may be the rate limiting factor in the regulation of collagen biosynthesis. Proline and hydroxyproline compose approximately 25% of collagen, which is the most common protein in the human body (21). Several studies suggested that prolidase activity is increased during oxidative stress in some diseases (23). It has been shown that prolidase activity is increased in many clinical diseases, such as Legg-Calve-Perthes disease (10), wound healing (24), chronic hepatic disease (25), erectile dysfunction (26), and hypertension (27), which indicates increased collagen turnover. Up until now, there is not study showing an increased prolidase activity in cases of CTS. Prolidase activity in patients with CTS were statitistically significantly higher compared with control This finding may indicates that increased collagen turnover and pathological changes occur in the subsynovial connective tissue in patients with CTS. Furthermore a positive correlation between prolidase activity and mSDL also D4M-D4U, whereas a negative correlation between prolidase activity and mSNCV was found. This finding also support role of prolidase activity in the pathogenesis of CTS. In the light of these findings, we concluded that there is a relation between increased serum prolidase activity and CTS, which may be interpreted as evidence of increased collagen turnover. Several limitations of the study should be considered. One potential limitation is the cross-sectional study design. In addition, the number of investigated patients with CTS was small. However, this is a preliminary study giving us an idea about the collagen metabolism in CTS by measuring serum prolidase activity. More comprehensive work and further clinical studies are needed to clarify the pathophysiological role of increased serum prolidase activity in CTS.

## References


1. *Cevik MU, Altun Y, Uzar E, Acar A, Yucel Y, Arikanoglu A, et al. Diagnostic value of F-wave inversion in patients with early carpal tunnel syndrome. Neuroscience letters. 2012; 508(2): 110-3. Epub 2012/01/05.*

2. *Manes HR. Prevalence of carpal tunnel syndrome in motorcyclists. Orthopedics. 2012; 35(5): 399-400. Epub 2012/05/17.*

3. *Ozturk N, Erin N, Tuzuner S. Changes in tissue substance P levels in patients with carpal tunnel syndrome. Neurosurgery. 2010; 67(6): 1655-60; discussion 60-1. Epub 2010/11/26.*

4. *Van Dijk MA, Reitsma JB, Fischer JC, Sanders GT. Indications for requesting laboratory tests for concurrent diseases in patients with carpal tunnel syndrome: a systematic review. Clinical chemistry. 2003; 49(9): 1437-44. Epub 2003/08/21.*

5. *Ettema AM, Amadio PC, Zhao C, Wold LE, O'Byrne MM, Moran SL, et al. Changes in the functional structure of the tenosynovium in idiopathic carpal tunnel syndrome: a scanning electron microscope study. Plastic and reconstructive surgery. 2006; 118(6): 1413-22. Epub 2006/10/20.*

6. *Oh J, Zhao C, Amadio PC, An KN, Zobitz ME, Wold LE. Immunolocalization of collagen types in the subsynovial connective tissue within the carpal tunnel in humans. Journal of orthopaedic research : official publication of the Orthopaedic Research Society. 2005; 23(5): 1226-31. Epub 2005/06/01.*

7. *Jinrok O, Zhao C, Amadio PC, An KN, Zobitz ME, Wold LE. Vascular pathologic changes in the flexor tenosynovium (subsynovial connective tissue) in idiopathic carpal tunnel syndrome. Journal of orthopaedic research : official publication of the Orthopaedic Research Society. 2004; 22(6): 1310-5. Epub 2004/10/12.*

8. *Duygu F, Koruk ST, Karsen H, Aksoy N, Taskin A, Hamidanoglu M. Prolidase and oxidative stress in chronic hepatitis C. Journal of clinical laboratory analysis. 2012; 26(4): 232-7. Epub 2012/07/20.*

9. *Gecit I, Aslan M, Gunes M, Pirincci N, Esen R, Demir H, et al. Serum prolidase activity, oxidative stress, and nitric oxide levels in patients with bladder cancer. Journal of cancer research and clinical oncology. 2012; 138(5): 739-43. Epub 2012/01/20.*

10. *Altay MA, Erturk C, Aksoy N, Taskin A, Bilge A, Celik H, et al. Serum prolidase activity and oxidative-antioxidative status in Legg-Calve-Perthes disease. Journal of pediatric orthopaedics Part B. 2011; 20(4): 222-6. Epub 2011/02/10.*

11. *Demirkol A, Uludag M, Soran N, Aksoy N, Gun K, Incebiyik S, et al. Total oxidative stress and antioxidant status in patients with carpal tunnel syndrome. Redox report : communications in free radical research. 2012. Epub 2012/10/24.*

12. *Myara I, Charpentier C, Lemonnier A. Optimal conditions for prolidase assay by proline colorimetric determination: application to iminodipeptiduria. Clinica chimica acta; international journal of clinical chemistry. 1982; 125(2): 193-205. Epub 1982/10/27.*

13. *Chinard FP. Photometric estimation of proline and ornithine. The Journal of biological chemistry. 1952; 199(1): 91-5. Epub 1952/11/01.*

14. *Ettema AM, Amadio PC, Zhao C, Wold LE, An KN. A histological and immunohistochemical study of the subsynovial connective tissue in idiopathic carpal tunnel syndrome. The Journal of bone and joint surgery American volume. 2004; 86-A(7): 1458-66. Epub 2004/07/15.*

15. *Moriya T, Zhao C, Cha SS, Schmelzer JD, Low PA, An KN, et al. Tendon injury produces changes in SSCT and nerve physiology similar to carpal tunnel syndrome in an in vivo rabbit model. Hand (N Y). 2011; 6(4): 399-407. Epub 2012/12/04.*

16. *Donato G, Galasso O, Valentino P, Conforti F, Zuccala V, Russo E, et al. Pathological findings in subsynovial connective tissue in idiopathic carpal tunnel syndrome. Clinical neuropathology. 2009; 28(2): 129-35. Epub 2009/04/10.*

17. *Oh J, Zhao C, Zobitz ME, Wold LE, An KN, Amadio PC. Morphological changes of collagen fibrils in the subsynovial connective tissue in carpal tunnel syndrome. The Journal of bone and joint surgery American volume. 2006; 88(4): 824-31. Epub 2006/04/06.*

18. *Van Doesburg MH, Mink van der Molen A, Henderson J, Cha SS, An KN, Amadio PC. Sonographic measurements of subsynovial connective tissue thickness in patients with carpal tunnel syndrome. Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine. 2012; 31(1): 31-6. Epub 2012/01/05.*

19. *Galasso O, Mariconda M, Donato G, Di Mizio G, Padua L, Brando A, et al. Histopathological, clinical, and electrophysiological features influencing postoperative outcomes in carpal tunnel syndrome. Journal of orthopaedic research: official publication of the Orthopaedic Research Society. 2011; 29(8): 1298-304. Epub 2011/03/15.*

20. *Kim JK, Koh YD, Kim JS, Hann HJ, Kim MJ. Oxidative stress in subsynovial connective tissue of idiopathic carpal tunnel syndrome. Journal of orthopaedic research: official publication of the Orthopaedic Research Society. 2010; 28(11): 1463-8. Epub 2010/09/28.*

21. *Vural M, Toy H, Camuzcuoglu H, Aksoy N. Comparison of prolidase enzyme activities of maternal serum and placental tissue in patients with early pregnancy failure. Archives of gynecology and obstetrics. 2011; 283(5): 953-8. Epub 2010/05/04.*

22. *Surazynski A, Miltyk W, Palka J, Phang JM. Prolidase-dependent regulation of collagen biosynthesis. Amino acids. 2008; 35(4): 731-8. Epub 2008/03/06.*

23. *Arioz DT, Camuzcuoglu H, Toy H, Kurt S, Celik H, Aksoy N. Serum prolidase activity and oxidative status in patients with stage I endometrial cancer. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society. 2009; 19(7): 1244-7. Epub 2009/10/14.*

24. *Oono T, Fujiwara Y, Yoshioka T, Arata J. Prolidase activity in chronic wound and blister fluids. The Journal of dermatology. 1997; 24(10): 626-9. Epub 1997/12/31.*

25. *Myara I, Myara A, Mangeot M, Fabre M, Charpentier C, Lemonnier A. Plasma prolidase activity: a possible index of collagen catabolism in chronic liver disease. Clinical chemistry. 1984; 30(2): 211-5. Epub 1984/02/01.*

26. *Savas M, Yeni E, Celik H, Ciftci H, Utangac M, Oncel H, et al. The association of serum prolidase activity and erectile dysfunction. Journal of andrology. 2010; 31(2): 146-54. Epub 2009/10/17.*

27. *Demirbag R, Yildiz A, Gur M, Yilmaz R, Elci K, Aksoy N. Serum prolidase activity in patients with hypertension and its relation with left ventricular hypertrophy. Clinical biochemistry. 2007; 40(13-14): 1020-5. Epub 2007/07/03.*

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# Comparison of clinical and microbiological effects of subgingival irrigation with chlorhexidine as an adjunct to mechanical therapy on pathologic periodontal pocket therapy

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**Abstract**

**Aim:** In this study, the effect of subgingival irrigation with chlorhexidine adjunct to scaling and root planing on clinical and microbiological parameters were evaluated. For this purpose, 40 patients which have at least 2 pathologic periodontal pockets with a probing depth greater than 5 mm have participated in this study.

**Material and Method:** Initial plaque index (PI), gingival index (GI), gingival bleeding index (GBI), probing depth (PD) and clinical attachment level (CAL) scores were recorded and subgingival plaque sample were taken for microbiological sampling. Spirochetes, cocci and nonmotil rods were scanned by light microscope. Patients randomly assigned to subgingival irrigation with chlorhexidine (0,2 %) and control groups. Irrigations were performed after scaling and root planing at first group. After one week, subgingival irrigations were performed again at first group. At control group only scaling and root planing was performed. Clinical recordings were repeated at 2 and 4 weeks and subgingival plaque sample were taken.

**Conclusion:** According to our results, significant improvement was shown on all clinical parameters at two therapy groups at 2 and 4 weeks. Intergroup comparision, clinical attachment level scores were significantly reduced in irrigation group. However no significant difference was found between groups. Positive improvements were shown on microbial flora at irrigation groups.

**Key words:** subgingival irrigation, chlorhexidine, mechanical therapy, pathologic, periodontal, pocket therapy

## Introduction

It is an accepted fact today that, microbial dental plaque is responsible for etiology of periodontal disease(1). After development of the idea of periodontal diseases are infectious diseases caused by specific micro-organisms, the researchers' attention focused on the use of antibacterial drugs as well as known treatment methods (2). Due to disadvantages of using systemic antibiotic treatments and to be understood that periodontal lesions resulted from microorganisms in the periodontal pocket, in order to increase the success of the treatments in the subgingival region, application of antimicrobial agents in topical way has come into question (3, 4). In order to ensure chemical plaque control, one of the methods provide the implementation of local antimicrobial agents is the irrigation process. Irrigation process is the most simple and practical method that and that allows the application of chemotherapeutic agents locally into the diseased area (5, 6).

The results of studies conducted to investigate the contributions of subgingival irrigation of periodontal treatment do not show accordance with each other. Some of these studies emphasize that, the subgingival irrigation process increased the success of conventional periodontal treatment methods, but some of them emphasize that, irrigation process did not have contribution that can be considered a positive for conventional therapy (5, 7, 8).

The most widely researched agent for subgingival irrigation is chlorhexidine. It has a broad antimicrobial spectrum and alcali structure. Chlorhexidine, at the same time, by binding to anionic acid groups in saliva glycoproteins, reduces the

formation of pelliculle and plaque colonization. In addition by binding to bacteria in the saliva, prevent them to hold the tooth surfaces (9, 10). As the reason for the unclear results obtained with subgingival irrigation with chlorhexidine, it has been suggested that serum proteins in the pocket reduce the antimicrobial potential of chlorhexidine instead of this agent does not have effect against the subgingival bacteria (11).

Starting from all the literature data, in our study, it has been aimed to compare the clinical and microbiological effects of subgingival irrigation with chlorhexidine as an adjunct to mechanical therapy in the patients with pathologic periodontal pocket.

## Material and methods

This study is carried out in Dicle University Faculty of Dentistry and Department of Periodontology and Dicle University Faculty of Medicine, Department of Microbiology. The study was performed with 28 male 12 female a total of 40 patients admitted to our clinic between the ages 24 and 61. The patients were informed about the procedures and their approvals were requested.

It has been taken care that, without discriminating age and gender, the presence of pathological periodontal pocket at least in 2 region of 5 mm or more, presence of at least 20 teeth in the mouth, no antibiotic treatment or periodontal therapy in the last six months, no condition of pregnancy or lactation, absence of systemic disease and allergy to the medication in patients.

Our study was carried out on two separate groups as I- Chlorhexidine and II- Control group. The individuals were placed into these groups randomly and all groups have been made to have 20 patients. As a solution of chlorhexidine, 0.2% chlorhexidine gluconate was used. During the study, plaque index (Modified Silness-Loe plaque Index (12)), gingival index (Loe-Silness (13)), gingival bleeding index (Ainamo and Bay (14)), pocket depth, clinical attachment level measurements were obtained from each cases and recorded in individual forms.

### *Microbiological Examination*

Sampling were performed followed by the measurement of pocket depth in the teeth detected. Prior to sampling, supragingival plaque on the tooth was removed. Then, a sterile Gracey curette was inserted into the pocket, and advanced to bottom of the pocket in the limit of pocket anatomy. Subgingival sample were put into saline solution in the sterile glass tubes. İt has been sent to the microbiology laboratory as soon as possible. A drop was taken from the material with sterile pasteur pipette and put on a clean slide and spread to form a thin layer. After drying it was detected by passing through flame. Preparations was staiened with 'Modified Gray' technique. Stained preparations were examined by a light microscope with immersion lens. Bacteria were classified in three different groups (Spirochetes, Cocci, and rods). Bacterial examination was carried out by the same person until the end of the study and three fiels were counted in each preperations (15).

### *Clinical Applications*

1. Measurement (0 Week): The initial measurement and the index evaluations of all patients included in the study were performed. Then, samples were taken for microbiological examination from the region which had the maximum pocket deepth. The impression were obtained from the patients in order to prepare occlusal stent. Initial periodontal therapy was performed. At the end of these processes, the experimental teeth were isolated with cotton rolls and irrigated for 5 minutes with the 10 ml of irrigating solution and this process was repated after one week. The control group was not undergone to irrigation process. The patients were given oral hygiene education.

2. Measurement (After 2 week): The oral hygiene information was reminded for patients if it was necessary. The entire measurement and index performed in the first session were repeated and the samples were taken for microbiological examination.

3. Measurement (After 4 week): The entire measurement, index and microbiological samples were taken again. During this period, the patients used any antibiotics were excluded from the study. If it was necessary, scalling and root planning was repeated for some patients.

### *Statistical Analysis*

For the statistical analysis of clinical data obtained, ANOVA and t-test (paired comparison) were

used. Calculations were perfomed with Minitab for Windows (ver: 11.00) statistical package program. For the statistical analysis of microbiological data and gingival bleeding index values, the chi-square test was used.

**Results**

The clinical parameters of the treatment groups in the stages of measurements were given in Table 1.

*Table 1. The mean and standard deviation of clinical parameters of the treatment groups in the stages of measurements*

| PI | Chlorhexidine | Control |
|---|---|---|
| 0 week | 2.800 ± 0.4104 | 2.800 ± 0.523 |
| 2 week | 1.250 ± 0.786 | 1.600 ± 0.681 |
| 4 week | 1.300 ± 0.733 | 1.400 ± 0.503 |
| GI | | |
| 0 week | 2.150 ± 0.3663 | 2.050 ± 0.2236 |
| 2 week | 1.550 ± 0.510 | 1.550 ± 0.510 |
| 4 week | 1.350 ± 0.489 | 1.300 ± 0.470 |
| PD (mm) | | |
| 0 week | 7.150 ± 1.424 | 6.200 ± 1.542 |
| 2 week | 5.600 ± 1.392 | 4.700 ± 1.625 |
| 4 week | 4.600 ± 1.536 | 3.900 ± 1.210 |
| CAL | | |
| 0 week | 8.700 ± 1.129 | 7.800 ± 1.281 |
| 2 week | 7.350 ± 1.182 | 6.750 ± 1.209 |
| 4 week | 6.450 ± 1.395 | 6.150 ± 1.226 |
| GBI (%) | | |
| 0 week | 100 | 100 |
| 2 week | 55 | 55 |
| 4 week | 35 | 35 |

The decreases in plaque index of two groups between 0 and 2 week; and 0 and 4 week were found to be significant. In the chlorhexidine group between 2 and 4 week, the value of plaque index increased but this raise was not found to be statistically significant.The decrease in the control group between 2 and 4 week was not found to be statistically significant. For the values of plaque index between 0, 2, and 4 weeks were not found to be significant between the groups.

The decreases in the values of gingival index were found to be significant for both groups but for 0, 2, and 4 weeks there was no significant difference between the groups.

There were significant decrease in pocket deepth values for both groups, a significant differences between the groups were not found.

The decreases obtaining in 0 and 2 week; and 0 and 4 week in the clinical attachment levels were found to be significant for both group. Between 2 and 4 week, only significant difference occured in the chlorhexidine group. 0 week clinical attachment level of the control group were significantly lower in the control group than chlorhexidine group ($F=3.17$ $p<0.05$ LSD= 0.7212). There was no significant difference between the groups for clinical attachment levels in 2 and 4 weeks.

The percentage of gingival bleeding index of the treatment groups were not found to be significant in three stage of measurements according to the chi-square test results.

*Table 2. The percentage of spirochetes, cocci and rods of the treatment groups in 3 stage of measurements (%)*

| Spirochetes (%) | Chlorhexidine | Control |
|---|---|---|
| 0 week | 36.33 | 27.50 |
| 2 week | 30.70 | 19.83 |
| 4 week | 28.56 | 14.70 |
| Cocci (%) | | |
| 0 week | 32.48 | 30.39 |
| 2 week | 43.20 | 43.43 |
| 4 week | 48.30 | 48.94 |
| Rods (%) | | |
| 0 week | 31.19 | 42.11 |
| 2 week | 26.10 | 36.74 |
| 4 week | 23.14 | 36.36 |

The percentage of spirochetes, cocci and rods of the treatment groups in 3 stage of measurements were given in Table 2.

Decreases in the percentages of spirochetes in each group were found to be statistically significant. It was found to be significant difference in the of three measurements between the groups according to the chi-square test ($\chi^2=87.998$ $p<0.001$). The percentage of spirochetes of the control group was significantly lower than the chlorhexidine group.

The increases of cocci values in the chlorhexidine and the control groups were statistically significant, however, there was no difference between the groups.

The decreases occured in the percentages of rods in the chlorhexidine group between 0 and 2

week, 0 and 4 week and 2 and 4 week were found to be statistically significant. The decreases occured in the percentages of rods in control group between 0 and 2 week, 0 and 4 week were found to be statistically significant. The rods percentage of 0. week of chlorhexidine group was significantly lower than the percentage of the control group ($\chi^2$=117.408 p<0.001).

## Discussion

Chlorhexidine is the one of the most researched agent using with the irrigation process which is a method providing application of local antimicrobial agents (16-21). However, the clinical and microbiological data obtained following chlorhexidine subgingival irrigation are not satisfactory (19, 22, 23). Based on these findings, in our study, in addition to the mechanical treatment of pathological periodontal pockets, the use of topical chlorhexidine irrigation was evaluated as the clinical and microbiological aspects.

According to the findings of our study similar to the literature, plaque index decreased in both chlorhexidine and control groups, but the significant differences between these two groups were not observed. This finding show that, an adequate oral hygiene alone could be effective in the control of plaque. With the obtain of recovery with fully implemented of scaling and root planning, it has been indicated that the importance of subgingival irrigation will not remain (22, 24). The results of studies with chlorhexidine, were parallel to our findings in terms of gingival index (17, 25). If we consider that, gingival index, largely is influenced primarily by plaque index, to not obtain difference between the grops in terms of plaque index explains this finding.

In our study, groups in terms of percentages of gingival bleeding index, the decreased were observed in both treatment groups. No statistically significant difference between groups was found. Similar to our results, some researchers determined that there were significant recovery obtained with probing but there were no difference between the groups (22, 24).

Several studies reported that, irrigation caused 1mm or less decrease in pocket depth (16, 23, 26, 27). On the other hand, if prior to irrigation, root planing is performed, decrease in pocket depth, becomes 2-3mm (17, 22-25). Therefore, it has been suggested that, for gingival recession and reduction in pocket deepth with clinical attachment gain, root planing needed to be done (28). The results obtained in this study were similar with these findings.

Other parameter used in the study is a clinical attachment level. According to the results, the initial attachment level of chlorhexidine irrigation group were significantly higher than the control group. Between 2 and 4 weeks there was no difference between the groups. The studies in the literature seem to support this view (17, 22).

As a result of microbiological examination in this study, the percentages of spirochetes decreased in both groups regularly at the stages of measurements. In previous studies, it has been identified that, there was a positive correlation between the plaque index values and percentage of subgingival spirochetes in microflora (29, 30). In our study, we think that the percentage of spirochetes of chlorhexidine irrigation group remains higher level than the other group may be related with the increase obtained in plaque index values in 4 week.

As a result of the implementation of subgingival irrigation as a monotherapy, a significant decrease was observed in microorganisms. However, it can not be completely eliminated, although a reduction in pathogens. Microorganisms, after short-term irrigation, return to their initial values in 1 to 8 weeks (23, 26). When root planing is applied before the irrigation, reduction and suppression of the bacteria are more and longer lasting (22- 25).

As a result of irrigation with chlorhexidine, it has been reported that, the suppression in spirochetes continued for 4 weeks before starting to rise towards the initial values (26). These results are paralel with our findings.

In our study, the increase in the percentage of cocci in both groups was statistically significant, this findings show similarity with the literature (16, 26).

In our study, the percentages of rods in the microbiological analysis, the value in the chlorhexidine group for 0 week was found to be the lowest, the decreases were obtained for both treatment group.

According to the findings of this study, the expected improvement was observed after treatment in both groups. However, irrigation group had no significant superiority compared to only scaling and root planing group.

The recent data are not sufficient to say that, one-time subgingival irrigation applied by the physician increases the length of efficicacy of the root planing. However, irrigations that the patients apply more often by themselves during the maintenance therapy may be useful. In addition, subgingival irrigation can be an important adjunct to conventional therapy for the patients who can not provide adequately the level of oral hygiene.

## References


1. *Socransky SS, Haffajee AD: The Bacterial Etiology of Destructive Periodontal Disease: Current Concepts. J. Periodontol. 1992; 63: 322-331.*

2. *Gordon JM, Walker CB: Current Status of Systemic Antibiotic Usage in Destructive Peridontal Disease. J. Periodontol., 1993; 64: 760-771.*

3. *Kornman KS: Controlled- Release Local Delivery Antimicrobials in Periodontics: Prospect for the Future. J. Periodontol. 1993; 64: 782-791.*

4. *Rosling BG, Slots J, Webber RL, Christersson LA, Genco RJ: Microbiological and Clinical Effects of Topical Subgingival Antimicrobial Treatment on Human Periodontal Disease. J. Clin. Periodontol., 1983; 10: 487-514.*

5. *Greenstein G: Effects of Subgingival Irrigation on Periodontal Status. J. Periodontol., 1987; 58: 827-836.*

6. *Newman HN: Modes of Application of Anti-plaque Chemicals. J. Clin Periodontol., 1986; 13: 965-974.*

7. *Goodman CH, Robinson PJ: Periodontal Therapy: Reviewing Subgingival Irrigations and Future Considerations. J. Am. Dent. Assoc., 1990; 121: 541-543.*

8. *Shiloah J, Hovious LA: The role of subgingival irrigation in the treatment of periodontitis. J. Periodontol. 1993; 64: 835-843.*

9. *Greenstein G, Berman C, Jaffin R: Chlorhexidine. an Adjunct to Periodontal Therapy. J. Periodontol., 1986; 57: 370-377.*

10. *Gjermo P: Chlorhexidine and Releated Compounds. J. Dent. Res., 1989; 68(Spec. Iss.): 1602-1608.*

11. *Wade WG, Addy M: In Vitro Activity of Chlorhexidine-Containing Mouthwash Against Subgingival Bacteria. J. Clin. Periodontol., 1989; 60. 511-515.*

12. *Sillness J, Löe H. Periodontal disease in pregnancy. Acta Odontol Scand 1964; 22: 121.*

13. *Löe H, Sillness J. Periodontal Disease in Pregnancy ( I ). Prevalence and Severity. Acta Odontol Scand 1963; 21: 533-551.*

14. *Ainamo J And Bay I. Problems and proposal for recording gingivitis and plaque. Int Dent J 1975; 25:229-235.*

15. *Quee TC, Bergeron MJ, Amsel R, Chan ECS: A Staining Method for Monitoring Subgingival Bacteria Associatted with Periodontal Disease. J. Periodont. Res., 1986; 21: 722-727.*

16. *Stabholz A, Nicholas AA, Zimmerman GJ, Wikesjö UME: Clinical and Antimicrobial Effects of a Single Episode of Subgingival Irrigation with Tetracycline HCL or Chlorhexidine in Deep Periodontal Pockets. J. Clin. Periodontol., 1998; 25: 794-800.*

17. *Shiloah J, Patters MR: DNA probe analyses of survival of selected periodontal pathogens following scaling, root planning and intra-pocket irrigation. J. Periodontol., 1994; 65: 568-575.*

18. *Lee MK, Ide M, Coward PY, Wilson RF. Effect of ultrasonic debridement using a Chlorhexidine irrigant on circulating levels of lipopolysaccharides and interleukin-6. J. Clin. Periodontol.,2008;35:415- 419.*

19. *Tenenbaum H, Luc E, Schaaf JF, Ducani MF, Cotton C, Elkaim R, Cuisinier FJG, Rogues C. An a 8-week randomized, controlled, clinical study of the use of a 0.1%chlorhexidine mouthwash by chronic periodontitis patients. J of İnvest. And Clin. Dentistry, 2011; 2: 29-37.*

20. *Duss C, Lang NP, Cosyn J, Persson GR. A randomized, controlled clinical trial on the clinical, microbiological, and staining effects of a novel 0.05% chlorhexidine/herbal extract and a 0.1% chlorhexidine mouthrinse adjunct to periodontal surgery. J Clin Periodontol 2010; 37: 988–997.*

21. *Guarnelli ME, Franceschetti G, Manfrini R, Trombelli L. Adjunctive effect of chlorhexidine in ultrasonic instrumentation of aggressive periodontitis patients: a pilot study. J Clin Periodontol 2008; 35: 333–341.*

22. *Oosterwaal PJM, Mikx FHM, Van't Hof MA, Renggli HH: Comparison of the Antimicrobial Effect of the Application of Chlorhexidine Gel, Amine Fluoride Gel and Stannous Fluoride Gel in Debrided Periodontal Pockets. J. Clin Periodontol., 1991; 18: 245-251.*

23. *Wennström JL, Dahlen G, Gröndahl K, Heijl L: Periodic subgingival antimicrobial irrigation of periodontal pockets. II- Microbiologic and radografic observation. J. Clin. Periodontol. 1987; 14: 573-580.*

24. *Braatz L, Garrett S, Claffey N, Egelberg J: Antimicrobial irrigation of deep pockets to supplement non-surgical periodontal therapy. II-Daily irrigation. J. Clin. Periodontol. 1985; 12: 630-638.*

25. *Southard SR: The effects of %2 chlorhexidine digluconate irrigation on clinical parameters and the level of Bacteriodes gingivalis in periodontal pockets. J. Clin. Periodontol., 1989; 60: 302-309.*

26. *Lander PE, Newcomb GM, Seymour GJ, Powell RN: The antimicrobial and clinical effects of a single subgingival irrigation of chlorhexidine in advanced periodontal lesions. J. Clin. Periodontol. 1986; 13: 74-80.*

27. *Vignarajah S, Newman HN, Bulman J, Pulsated Jet Subgingival Irrigation with 0,1% Chlorhexidine, Simplified Oral Hygiene and Chronic Periodontitis. J. Clin. Periodontol., 1989; 16: 365-370.*

28. *Greenstein G: Supragingival and Subgingival Irrigation: Practical Application in Treatment of Periodontal Diseases. Comp. Contin. Educ. Dent., 1991; 13: 1098-1125.*

29. *Listgarten MA, Levin S: Positive Correlation between the Proportions of Subgingival Spirochetes and Motile Bacteria and Susceptibility of Human Subjects to Periodontal Deterioration. J. Clin. Periodontol., 1981; 8: 122-138.*

30. *Omar AA, Newman HN, Bulman J, Osborn J, Associations between Subgingival Plaque Bacterial Morphotypes and Clinical İndices. J. Clin. Periodontol., 1991; 18: 555-566.*

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# Brucella should be included in the differential diagnosis of infective endocarditis in endemic regions

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## Abstract

**Background:** Evaluation of the clinical and laboratory characteristics of patients with Infective Endocarditis (IE) was studied among patients treated at a clinic in Turkey to determine the factors associated with IE that affect hospital mortality.

**Methods:** During a four-year period, 61 patients were treated and followed for IE. The diagnosis of IE was made according to the Duke Criteria.

**Results:** The most common complaint on presentation was fever (82.2%). All patients underwent transthoracic echocardiography; transesophageal echocardiography was performed in 39 patients (63.9%). Rheumatic heart disease (50.8%) was the most common preexisting risk factor for IE. The incidence of native valve involvement (57.4%) was greater than for prosthetic valve (34.4%) involvement. The blood cultures were negative in 22 patients. Brucella IE was detected among eight (13.1%) patients in the culture positive group. According to clinic data, echocardiography and a standard agglutination test titer of ≥1/160 were associated with the detection of Brucella IE among eight patients in the culture negative group. Brucella IE was observed in 26.2% of patients. Mortality was observed in patients that developed congestive heart failure (100%).

**Conclusion:** Diagnosis and treatment of IE should include a differential diagnosis that takes into consideration the epidemiology and clinical features of IE in a given community. Compared to Western countries, IE in this cohort occurred in a relatively younger population, with rheumatic heart disease as the most common underlying heart disease. In addition, IE associated with Brucellosis should be considered in regions where Brucellosis is endemic.

**Key words:** Infective Endocarditis, Brucella, Hospital Mortality.

## Introduction

Infective Endocarditis (IE) is a microbial infection of cardiovascular structures, intrathoracic veins of the heart or intra-cardiac devices (e.g. prosthesis valves and cardiac pacemakers) that are directly exposed to the blood stream (1). The most common organisms associated with IE are streptococci; S. aureus is the second most common (2). Complications occurred in 50-60% of patients with IE during the 1970s; currently, 40-50% of patients develop medical or surgical complications (3). The most significant complications include cardiac failure, embolic events, renal failure, cerebral events and paravalvular abscess formation (3). Mortality has been reported in 15-20% of affected patients (4).

Brucellosis is a zoonosis with worldwide reach; it is especially prevalent in the Mediterranean Basin, Mexico and the Arabian Peninsula (5). It is a systemic infectious disease that potentially affects almost every organ. Cardiovascular complications, which include pericarditis, endocarditis and myocarditis, occur in less than 2% of affected patients (6). Endocarditis, although a rare complication of brucellosis, is the main cause of death from this disease. Brucella endocarditis is often culture negative; it accounts for a number of cases of pathogen-induced endocarditis with negative blood cultures (7). Blood cultures have a low sensitivity (15-20%) due to the slow growth rate of Brucella spp (8).

In the current study, the aim was to review the clinical and laboratory characteristics of patients diagnosed with IE to determine the factors associated with hospital mortality.

## Methods

During a four-year period at a clinic in Turkey (October 2006/2010), 61 patients (33 male, 28 fe-

male; median age 44±18) were followed and treated for IE. The clinical characteristics (age, gender, hemodynamic properties, monitoring period and mortality), medical background (e.g. cardiac valve disease, history of cardiac surgery, congenital heart disease, systemic vascular disease, undergoing dialysis, and intravenous drug dependency), physical properties (e.g. cardiac murmur, mechanic cardiac valve sound, Osler nodules, Janeway lesions, and Roth spots), and echocardiographic features (e.g. valve narrowing and/or failure, valve prolapses, rheumatic or degenerative changes, prosthesis valve dysfunction, cardiac failure, vegetation, abscess, and congenital heart disease) were determined. The Ethics Committee at Yuzuncu Yil University approved this study.

Patients were diagnosed according to a modified Duke Criteria (9). Brucella endocarditis was confirmed by endocarditis findings associated with either Brucella spp. positive blood cultures or a standard agglutination test (SAT) titer ≥1/160. The agglutination test was performed using a commercial kit (Cromatest, Knickerbacker Laboratories, Barcelona, Spain).

### *Statistics*

Data were analyzed using SPSS 10.0 (SPSS Inc., Chicago, Illinois, USA). The results are expressed as the mean ± standard deviation or percentage.

## Results

Sixty one patients, with a mean age of 44±18 years included 33 males (54%) and 28 females (46%) were enrolled in this study. The presenting complaints of patients referred are shown in Table 1. The mean duration from the beginning of symptoms in patients to arrival to the hospital was 2.5 ± 1 weeks (the earliest was during the first week and the latest was during the fifth week). Electrocardiography demonstrated sinus rhythm in 45 patients (73.8%), atrial fibrillation in 15 patients (24.6%) and pace rhythm in 1 patient (1.6%). The most frequently encountered laboratory abnormalities were high sedimentation rates and C-reactive protein (CRP) levels, leukocytosis and anemia (75.4%, 70.5%, 68.9% and 65.6% respectively). Rheumatic heart disease and prosthetic heart valve disease were significant predis- posing factors associated with IE; other associated factors are listed in Figure 1.

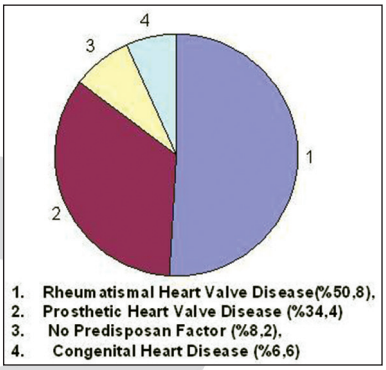


*Figure 1. Distribution of underlying heart disease in the patients diagnosed with Infective Endocarditis*

The blood cultures of 22 patients (36.1%) that were diagnosed with IE were negative, while 31 patients (50.8%) had positive blood cultures. Brucella IE was detected in eight (13.1%) patients in the culture positive group. According to clinic data, echocardiography and Brucella standard agglutination test (SAT) titers of ≥1/160 were asso-

*Table 1. Symptoms of patients during referral*

| | Female (n: 28) | Male (n: 33) | Total (n: 61) |
|---|---|---|---|
| Fever | 21 | 29 | 50 (82%) |
| Fatigue | 20 | 25 | 45 (73.8%) |
| Shortness of breath | 19 | 15 | 34 (55.7%) |
| Loss of appetite | 15 | 14 | 29 (47.5%) |
| Weight loss | 11 | 9 | 20 (32.8%) |
| Coughing | 10 | 6 | 16 (26.2%) |
| Head ache | 6 | 6 | 12 (19.7%) |
| Chest pain | 5 | 5 | 10 (6.4%) |
| Joint pain | 4 | 2 | 6 (9.8%) |
| Stroke | 1 | 1 | 2 (3.3%) |

*Table 2. Distribution of blood culture results*

| | Natural valve | | Mechanic valve | | Other (Lead, congenital) | |
|---|---|---|---|---|---|---|
| | Number | Percent | Number | Percent | Number | Percent |
| Mssa | 5 | 8.2 | 4 | 6.6 | 1 | 1.6 |
| Mrsa | 4 | 6.6 | 3 | 4.9 | 1 | 1.6 |
| S.epidermis | - | | 1 | 1.6 | - | |
| Streptococcus | 4 | 6.6 | 4 | 6.6 | 1 | 1.6 |
| Brucella | 5 | 8.1 | 2 | 3.3 | 1 | 1.6 |
| Enterococci | 1 | 1.6 | 1 | 1.6 | - | |
| HACEK | - | | 1 | 1.6 | - | |
| Negative culture | 18 | 29.5 | 4 | 6.6 | 0 | 0 |
| Total | 35 | 57.4 | 21 | 34.4 | 5 | 8.1 |

*(Mssa: Methicillin-susceptible Staphylococcus aureus, Mrsa: Methicillin -resistant Staphylococcus aureus, HACEK: Haemophilus, Actinobacillus, Cardiobacterium, Eikenella, Kingella)*

ciated with Brucella IE in eight (13.1%) patients, in the culture negative group. In the culture negative group, Brucella IE was observed among eight (36.4%) patients. Brucella IE was detected in 16 (26.2%) patients. The median time for blood culture detection of Brucellosis was seven weeks. The culture results of patients and their distribution are shown in Table 2.

Thirty-seven patients (60.7%) had a history of antibiotic usage prior to their hospitalization. Transthoracic echocardiography (TTE) was performed in all patients. Vegetations were observed in 51 of the patients (83.6%) that underwent TTE. Trans esophageal echocardiography was performed in 39 patients (63.9%). Mitral valve involvement was the most common finding; echocardiography findings of the patients are listed in Table 3.

Patients received antibiotic therapy for a mean of 5.7±1.5 (2.5-10) weeks. Antibiotic therapy was administered to 34 patients (55.7%) while 27 of the patients (44.3%) received surgical intervention plus antibiotic therapy. Prosthetic valve replacement was performed in 19 of the patients (31.1%) that had surgical intervention, four patients (6.6%) received valve restoration and four patients (6.7%) had other surgical procedures. The mean duration of surgical intervention was calculated as 7.9 ± 11 (0-42) days, calculated from the moment the patient was hospitalized. The mean duration of hospitalization was 40 ± 10.5 days.

Hospital mortality was observed in 10 patients (16.4%). Mortality occurred in patients with congestive heart failure (100%) and brucellosis (in 5 patients, 50%). The most common complication was heart failure; the distribution of complications is shown in Table 4.

*Table 3. Echocardiograph findings and valve involvement*

| M mode and 2D Echocardiography Findings B | |
|---|---|
| Left ventricular diastole end diameter (mm) | 54±9 |
| Left ventricular systole end diameter (mm) | 39±7 |
| Sol atrium diameter (mm) | 44±7 |
| Interventricular septum diameter (mm) | 12±3 |
| Left ventricular posterior wall diameter (mm) | 11±3 |
| Left ventricular ejeksiyon fraction (%) | 50±16 |
| **Valve involvement** | |
| Native valve | 35 (57.4%) |
| Mitral | 18 (29.5%) |
| Aorta | 11 (18%) |
| Aorta and mitral | 4 (6.6%) |
| Tricuspid | 2 (3.3%) |
| Prosthesis valve | 21 (34.4%) |
| Mitral | 10 (16.4%) |
| Aorta | 7 (11.5%) |
| Aorta and mitral | 3 (4.9%) |
| Tricuspid | 1 (1.6%) |

## Discussion

In the current study patients that were diagnosed with and followed for IE at a clinic in Turkey over four years were studied. The mean age at diagnosis, of IE, was reported as 53±16 by Netzer et al (10), 60±16 years by Habib et al (11), 45±16 years by Sucu et al (12) and 47±17 years by Inanc et al (13). In

*Table 4. Complications and their distribution*

| | Native valve n: 35 | Prosthesis valve n: 21 | Other[1] n: 5 |
|---|---|---|---|
| Cardiac failure | 9 (25.7%) | 12 (57.1%) | 1 (20%) |
| Renal complications | 4 (11.4%) | 4 (19%) | 0 (0%) |
| Cerebral complications | 0 (0%) | 1 (4.8%) | 0 (0%) |
| Paravalvular abscess | 1 (2.9%) | 2 (9.5%) | 0 (0%) |
| Leaflet/cuspis perforation | 2 (5.7%) | 0 (0%) | 0 (0%) |
| Shock table | 1 (2.9%) | 2 (9.5%) | 0 (0%) |
| AV block | 1 (2.9%) | 0 (0%) | 1 (20%) |
| Hospital mortality | 4 (11.4%) | 6 (28.6%) | 0 (0%) |

*[1]: Pace lead and congenital heart disease (Atrial Septal Defect, Ventricular Septal Defect)*

this study the mean age at diagnosis of IE was 44±18 years. This finding shows that IE was observed at an earlier age in this country compared to Western communities. The underlying cause of IE observed at earlier ages, might be associated with rheumatic cardiac valve disease, which played an important role as a significant predisposing risk factor.

In the current study, laboratory parameters were compatible with prior data including anemia, leukocytosis and high ESR and CRP levels. In two separate studies, anemia and leukocytosis were found in 50-70% of the patients. In a study carried out by Inanc et al, high CRP levels and ESR were reported in 81% and 78%, respectively (13). The most common laboratory abnormalities in this study were a high ESR and CRP levels; a high ESR level in 75.4% and high CRP level in 70.5%. Rheumatic cardiac valve disease has decreased in developed countries but remains a serious concern in Turkey (14). In this study, 57.4% of the patients had native valves involved while 34.4% had prosthetic valve endocarditis. The most common cardiac risk factor for the development of IE (50.8%) was cardiac valve disease associated with ARF.

Blood cultures are important to both confirm the diagnosis of IE as well as to determine a therapeutic strategy (15). In a study carried out by Tugcu et al (16), positive blood cultures were found in 58.8% of patients, while 56% were positive in a study reported by Inanc et al (13). A negative blood culture may be due to prior antibiotic use. In this study, there was a positive history of antibiotic use among 60.7% of the patients. The blood cultures were negative in 22 patients (36.1%) that were diagnosed with IE; the blood cultures were positive in 31 patients (50.8%).

In a study of 329 patients, reported by Cabell et al, the frequency of S. aureus increased during recent years; IE cases associated with S. viridans has decreased (2). Cay et al reported that Staphylococcus was the most common organism (33.3%) associated with IE in Turkey (17). In this study, the most common organism identified was Staphylococcus (48.7%) and Streptococcus (23.1%) was the second most common.

In addition, this geographic region is endemic for Brucellosis. Even though endocarditis associated with Brucella infection is a rare complication, the frequency of endocarditis associated with Brucellosis is increased in regions where Brucellosis is endemic. Brucella IE was detected in eight (13.1%) patients in the culture positive group. According to clinic data, echocardiography and a Brucella standard agglutination test (SAT) titer ≥1/160 were associated with Brucella IE among eight (13.1%) patients in the culture negative group. In the culture negative group, Brucella IE was observed in eight (36.4%) patients. Brucella IE was observed in 16 (26.2%) patients, in total.

The clinical characteristics of patients diagnosed with Brucella Endocarditis were investigated as well as complications, relapse rates and mortality; these rates were found to be higher when compared to other IE cases (18). In this series, at least one complication was found in all of the patients diagnosed with Brucella Endocarditis. Three patients died due to complications.

In the present study, TTE was performed in all patients and vegetations were observed in 83.6% of the patients. In a study carried out by Cay et al, vegetations were detected in 71.9% of the patients (17). Transesophageal echocardiography provides

important diagnostic information in doubtful cases of IE. Especially with regard to prosthetic valves, TEE was more sensitive than conventional TTE for identification of intra-cardiac vegetations (60-65% versus 95%) (19). In the present study, TEE was performed in 63.9% of the patients and vegetations observed in 87.2% of these patients. Similarly, in a study carried out by Ozveren et al (20), TEE was performed in 61% of the patients and vegetations were reported in 97% of these patients.

Complications associated with IE were observed in 50-60% of cases during the 1970s and have decreased to 40-50% currently due to medical and surgical interventions (3). The most common complications observed are cardiac failure and renal failure (3). In a study carried out by Sucu et al (12), the most common complication was cardiac failure, found in 31.9% of cases. Tugcu et al (21) reported that cardiac failure was the most common complication (55.9%). In this study, the most common complication was cardiac failure (36.1%).

Treatment of IE continues to be a challenge. Ozveren et al (20) reported that 24% of patients received only antibiotic therapy, while 76% of the patients received antibiotic therapy plus surgical intervention; the mortality was reported as 8%. In another study carried out by Tugcu et al (16) 39.7% of the patients received medical therapy, while 60.3% of patients had surgical intervention; the mortality rate was reported as 25%. In this study, 55.7% of the patients received only antibiotic therapy, while 44.3% of the patients received antibiotic therapy and had surgical intervention. The mean duration of antibiotic therapy was 5.7±1.5 weeks and the mortality rate was 16.4%.

Compared to the United States and Europe, the IE observed in this study included younger patients, the frequency was higher among females and the most significant predisposing factor was rheumatic heart disease. There was an increase in the number of cases of IE in regions where Brucellosis was endemic. Brucellosis should be included in the differential diagnosis of IE, especially in geographic regions where it is endemic.

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**References**

1. *Fink AM. Endocarditis after valve replacement surgery. Early recognition and treatment are essential to averting deadly complications. Am J Nurs 2006; 106: 40-51.*

2. *Cabell CH, Jollis JG, Peterson GE, et al. Changing patient characteristics and the effect on mortality in endocarditis. Arch Intern Med. 2002 Jan 14; 162: 90-4.*

3. *Mills J, Utley J, Abbott. Heart failure in infective endocarditis. Chest 1974 ; 66: 151-9*

4. *Tornos P, Lung B, Permanyer-Miralda G, et al. Infective endocarditis in Europe: Lessons from the Euro heart surgery. Heart 2005; 91: 571-575*

5. *Keles C, Bozouls N, Sismanoglu M, et al. Surgical treatment of Brucella endocarditis. Ann Thorac Surg 2001; 71: 1160–3*

6. *Colmenero JD, Reguera JM, Martos F, et al. Complications associated with Brucella melitensis infection: a study of 530 cases. Medicine 1996; 75: 195–211*

7. *Berbari EF, Cockerill FR, Steckelberg JM. Infective endocarditis due to unusual or fastidious microorganisms. Mayo Clin Proc 1997; 72: 532–42*

8. *Fernandez-Guerrero ML. Zoonotic endocarditis. Inf Dis Clin N Am 1993; 7: 135–52*

9. *Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications: a statement for healthcare professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on cardiovascular Disease in the Young, and the Council on Clinical Cardiology, Stroke, and Cardiovascular Surgery and Anesthesia, AHA: endorsed by the Infectious Diseases Society of America. Circulation 2005; 111: 394-434*

10. *Netzer RO, Altwegg SC, Zollinger E, Tauber M, Carrel T, Seiler C. Infective endocarditis: determinants of long term outcome. Heart 2002; 88: 61-66.*

11. *Habib G, Tribouilloy C, Thuny F, et al. Prosthetic valve endocarditis: who needs surgery? A multicenter study of 104 cases. Heart 2005; 91: 954-959*

12. *Sucu M, Davutoğlu V, Ozer O, M.D, Aksoy M. Epidemiological, clinical and microbiological profile of infective endocarditis in a tertiary hospital in the South-East Anatolia Region. Turk Kardiyol Dern Ars -Arch Turk Soc Cardiol 2010; 38: 107-111-7*

13. *İnanc T, Kaya M, Kaya E, et al. İnfektif Endokardit: retrospektif olarak 27 hastanın değerlendirilmesi. Tıp Araştırmaları Dergisi 2007: 5: 91-99*

14. *Yavuz SS, Eren M, Yavuz A, et al. İnfektif endokardit: 58 olgunun değerlendirilmesi. Klimik dergisi 2003; 16: 55-62*

15. *Becson PB, Brannon ES, Warren JV. Observations on the sites of removal of bac teria from the blood of patients with bacterial endocarditis. J Exp Med 1945; 81: 9-23.*

16. *Tuğcu A, Yıldırımturk O, Baytaroğlu C, et al. Clinical spectrum, presenta tion, and risk factors for mortality in infective endocarditis: a review of 68 cases at a tertiary care center in Turkey. Turk Kardiyol Dern Ars - Arch Turk Soc Cardiol 2009; 37: 9-189*

17. *Cay S, Gurel O, Korkmaz S. Enfektif endokarditli olguların klinik ve epidemiyolojik ozellikleri. Turk Kardiyol Dern Ars - Arch Turk Soc Cardiol 2009; 37: 182-186*

18. *Gunes Y, Tuncer M, Guntekin U, et al. Clinical characteristics and outcome of Brucella endocarditis. Trop Doct. 2009; 39: 85-8.*

19. *Erbel R, Rohmann S, Drexler M, et al. Improved diagnostic value of echocardio graphy in patients with infective endocarditis by transesophageal approach: a prospeetive study. Eur Heart J. 1988; 9: 43-53.*

20. *Ozveren O. İnfektif endokardit olgularında klinik, laboratuar ve ekokardiyografik parametrelerin orta-uzun sureli izlem sonuçları. İstanbul – 2004; 56.*

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# Substance P mediates the influence of chronic unpredictable stress on a rat model of asthma

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## Abstract

**Objectives:** The aims of the study were to detect the influence of the chronic unpredictable stress (CUS) on asthma and the role of substance P in the mediation of the CUS effects on a rat model of asthma.

**Methods:** Three groups of Wistar rats were sensitized with ovalbumin (OVA) by intraperitoneal injection on day 1 and day 7 and were repeatedly challenged with OVA aerosol via the airways from day 15. Two groups of the OVA-sensitized and challenged rats were housed and exposed to a CUS procedure starting from day 44. One group of the stressed asthmatic rats was given NK-1 receptor antagonist from day 44. Additionally, 10 Wistar rats were taken as the control group. Numbers of total leukocytes and differentiation in bronchoalveolar lavage fluid (BALF) were determined. Levels of neuropeptide substance P and cytokines IL-1β, IL-4, TGF-β1 were measured using ELISA.

**Results:** Application of CUS to asthmatic rats resulted in a significant increase of the leukocytes numbers in BALF. Levels of substance P increased in the stressed asthmatic rats as compared with those of the non-stressed asthmatic animals. Furthermore, stressed asthmatic rats had higher levels of TGF-β1, IL-1βand IL-4 than the unstressed asthmatic animals. The increases of inflammatory cells, TGF-β1, IL-1β and IL-4 were partially blocked by NK-1 receptor antagonist.

**Conclusions:** CUS exacerbated asthma and substance P might contribute to the pathogenesis of the exacerbation.

**Key words:** Substance P, chronic unpredictable stress (CUS), rat, asthma, airway inflammation

## Introduction

By clinical observation and epidemiological research, psychological stress has long been recognized to be associated with increased risk of asthma incidence, increased symptom severity and more healthcare utilization (1-8). However, conclusive experimental evidence is still limited and little is known about the underlying mechanisms.

Some kinds of stress have been applied to animals to detect the role of the stress in asthma exacerbations. Joachim RA exposed the OVA-sensitized and challenged mice to sound stress for a single 24-hour period, and found the sound stress enhanced airway reactivity and increased leukocyte numbers in BAL fluids (9). Okuyama K suggested that the acute restraint stress for 6 hours modified the allergic airway responses and the chronic restraint stress induced exacerbation of allergic airway inflammation in asthmatic murines (10). Portela CP's study showed that the repeated foot shocks stress could affect smooth muscle tonus around the airways in OVA-sensitized rats (11). In those studies, the animals received either acute stress or unimodal repeated stress, which is unlike the asthmatic patients who usually undergo many kinds of chronic stress from daily hassles, sufferings from the disease and treatment failure. It is generally accepted that different kinds of stress may produce different influence on body. To date, the influence of chronic unpredictable stress on airway inflammation and cellular damage in asthmatic animals still remains unclear.

Although researchers around the world have tried to explain the mechanisms linking psychological stress to asthmatic responses, the precise mechanisms are still not well understood (12). Animal studies showed that substance P has been shown to play an important role in airway inflammation and

airway injury (13-16). NK-1 receptor blockade could reduce lung inflammation and injury (17-19). A clinical study measured plasma substance P levels in patients with both asthmatic and non-asthmatic cough and concluded that increased levels of substance P might be related to airway sensitivity (20). Zhang D also found asthmatic patients had higher substance P levels in plasma than the healthy subjects (21). Yaraee R's study showed that substance P significantly augments TGF-β production of both BEAS and A54 cells and concluded that substance P can directly modulate the release of TGF-β from human bronchial epithelial cell line (22). Substance P was also found to stimulate human airway submucosal gland secretion mainly via a CFTR-dependent process (23). Furthermore, substance P, which is supposed to be released into tissues in response to stress, is involved in the pathogenesis of stress-related disorders, such as depression and anxiety (24-27). To our knowledge, only two studies detected the changes of substance P induced by stress in asthmatic animals. A study showed that substance P level significantly increased in plasma and bronchoalveolar lavage fluid, but significantly decreased in bronchial tissue after electric shock stress in biphasic asthma-responsive guinea pigs (28). Joachim RA found that the sound stress (applied to the animals for 24 h) led to the stimulation of substance P expression in airway-specific neurons in OVA sensitized mice (29). In another study, NK-1 receptor has been shown to play an important role in mediating the acute sound stress effects in allergen-induced airway inflammation (30). But the influence of chronic unpredictable stress on substance P in asthmatic animals and the role of substance P in the mediation of the CUS effects on asthma still remain unknown.

Therefore, the purpose of this study was to explore the influences of chronic unpredictable stress on asthma, and to test the changes of substance P level in the asthmatic animals after CUS exposure.

## Materials and Methods

### *Experimental animals and preparation*

All animals used in this study were 4-week-old male Wistar rats (Shandong University, China) and all procedures performed on the animals were in compliance with the Chinese Council of Animal Care guidelines (approved by the Ethics Committee for Animal Experimentation of Weifang Medical University).

Forty Wistar rats were divided into four groups (10 rats in every group): control group, non-stressed asthmatic group, stressed asthmatic group and treatment group at random. Rats except the control group were sensitized and challenged with chicken OVA (Sigma, USA) and 200 mg of aluminum hydroxide (Sigma, USA) in 1 ml of sterile saline. The sensitized rats were exposed to 20-minute of 1% OVA aerosol (40 ml/20 min) every day from day 15 to day 43 using an Ultrasonic nebulizer, followed by a chronic challenge phase which consisted of one exposure every 5 days till the end of the experiment. The control rats were treated with a sterile saline intraperitoneal injec-

*Table 1. Chronic unpredictable stress procedure*

| | Week 1 | Week 2 | Week 3 | Week 4 |
|---|---|---|---|---|
| Monday | 4° C cold swimming (5 min) | 45° C oven (5 min) | Restraint stress 1 h, | 4° C cold swimming (5 min) |
| Tuesday | Sound stress (75 dB) (2 h) | Cage tilt (24 h) | Lights on overnight | Tail pinch (1 min) |
| Wednesday | Tail pinch (1 min) | Food deprivation (24 h) | 24 h wet cage | lights off (6AM-18PM) |
| Thursday | 45° C oven (5 min) | Food deprivation (24 h) | 45° C oven (5 min) | Water deprivation (24 h) |
| Friday | 24 h wet cage | lights off (6AM-18PM) | Tail pinch (1 min) | Water deprivation (24 h) |
| Saturday | Lights on overnight | Tail pinch (1 min) | Sound stress (75 dB) (2 h) | Cage tilt (24 h) |
| Sunday | Restraint stress 1 h, | 4° C cold swimming (5 min) | 4° C cold swimming (5 min) | 45° C oven (5 min) |

tion for sham sensitization and challenged with sterile saline. From day 44, rats of stressed asthmatic group and treatment group were exposed to a CUS procedure which lasted for 28 consecutive days and consisted of one stressor in a random order per day. Stressors that are widely used in the literature and were previously utilized in published work were selected (31-33) (Shown in table 1). The animals in the treatment group orally received NK1 receptor antagonist aprepitant 10 mg/kg/day for 28 days from day 44. The control rats remained undisturbed during the CMS procedure.

***Inflammatory cell counts in BALF***

To evaluate the airway inflammation, rats were deeply anesthetized and sacrificed after the last stress stimulation. The right lung of each rat was lavaged with 3 successive 3 ml volumes of saline instilled by syringe. Then, 1 ml of the lavage fluid was centrifuged at 1500 rpm for 10 min. The sediment was immediately resuspended in 1 ml of saline. Cells in the lavage fluid were stained with Wright-Giemsa stain, the total leukocytes counts were determined and differential cell counts were performed under light microscope.

***P substance measurement***

Lung tissue was removed and homogenizer was used for preparation of the tissue homogenate. The tissue homogenate was centrifuged at 1500 rpm for 10 min. Plasma was collected after the centrifugation of the 2 milliliters of blood. The contents of substance P in plasma and lung tissue homogenate were determined using ELISA according to the manufacturer's instructions (R&D Systems, USA).

***Cytokines measurement***

Levels of IL-1β and IL-4 in BALF, as well as TGF-β1 in BALF and lung tissue homogenate were measured using Elisa (R&D Systems, USA).

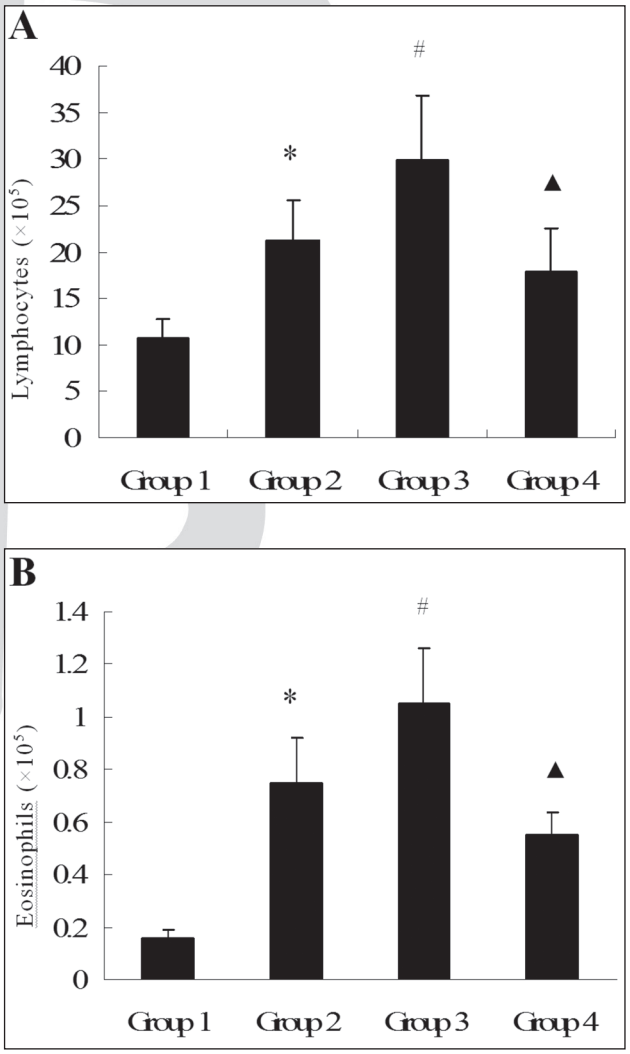
***Statistical analysis***

The analyses were carried out using SPSS 13.0. All the data were expressed as mean ± SD. One-way analysis of variance (ANOVA) followed by Scheffe test was used to determine the significant difference among multiple groups. P-values < 0.05 were considered statistically significant.

**Results**

***BALF cells assessments***

As shown in figure 1, sensitization and challenge with OVA significantly increased a total number of leukocytes ($p<0.05$), as well as numbers of macrophages ($p<0.05$), eosinophils ($p<0.05$) and lymphocytes ($p<0.05$) in BALF of the non-stressed asthmatic rats compared to those of the control rats. CUS further induced greater numbers of total leukocytes ($p<0.05$), eosinophils ($p<0.05$) and lymphocytes($p<0.05$) in BALF of the stressed asthmatic rats in comparison with the non-stressed asthmatic animals, while no significant difference of number of macrophages between the two groups were observed ($p>0.05$). Application of aprepitant significantly decreased numbers the inflammatory cells (all $p<0.05$). The data suggested that CUS exacerbated the airway inflammation in asthmatic rats, whereas the exacerbation could be blocked by treatment with the NK-1 receptor antagonist.



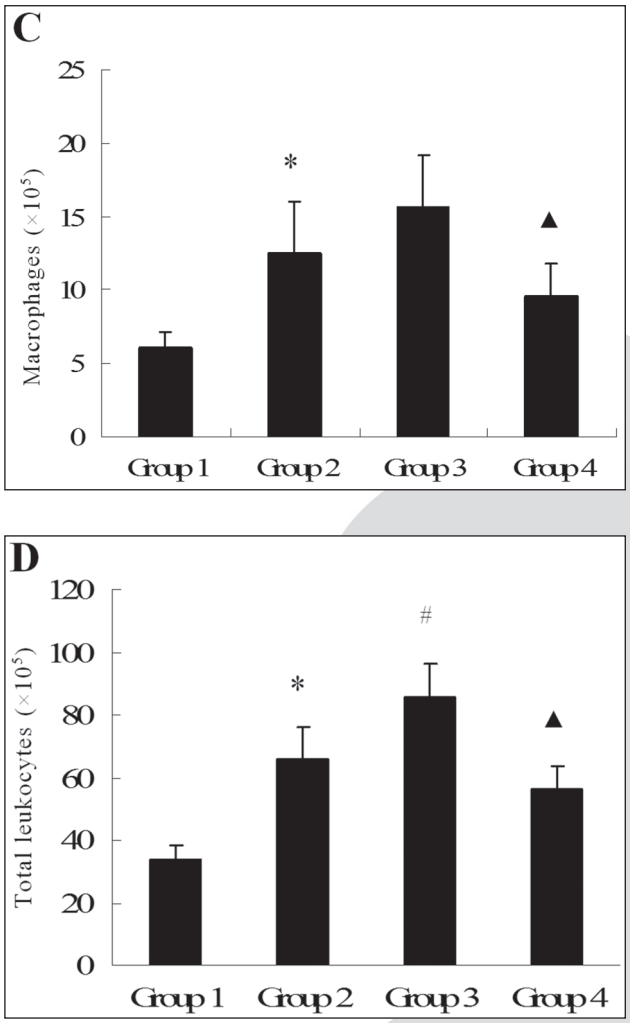



*Figure 1. Total and differential BALF cell counts*

*BAL fluid was collected and total leukocytes counts were determined and differential cell counts were performed under light microscope. A: Lymphocytes, B: Eosinophils, C: Macrophages, D: Total leukocytes. Data were presented as mean ± SD. * P＜0.05 vs. Group 1; # P＜0.05 vs. Group 2; ▲P＜0.05 vs. Group 3.*

*Note: Group 1: control group; Group 2: non-stressed asthmatic group; Group 3: stressed asthmatic group; Group 4: treatment group.*

### *P substance assessments*

Significantly elevated substance P levels in plasma and lung tissue of the stressed asthmatic rats were observed as compared with those of the non-stressed asthmatic rats (both p<0.05). No significant difference of substance P levels was found between the stressed asthmatic rats and the rats receiving NK-1 receptor antagonist (p>0.05). (Figure 2)

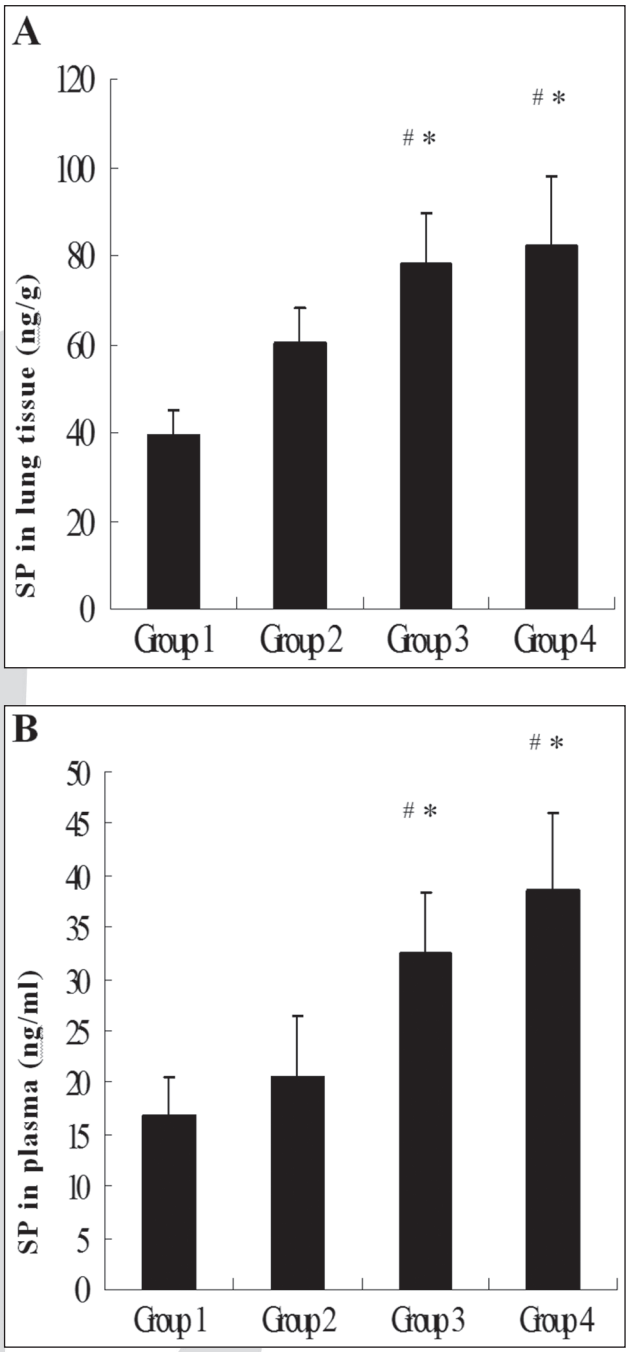



*Figure 2. Levels of substance P in lung tissue and plasma*

*Levels of substance P in lung tissue (A) and plasma (B) were determined using ELISA. Data were presented as mea ± SD. * P＜0.05 vs. Group 1; # P＜0.05 vs. Group 2; ▲P＜0.05 vs. Group 3.*

*Note: Group 1: control group; Group 2: non-stressed asthmatic group; Group 3: stressed asthmatic group; Group 4: treatment group.*

### *TGF-β1 assessments*

We measured the levels of TGF-β1 which is a cytokine involved in pathogenesis of the airway remodeling. We found that its levels of the non-stressed asthmatic rats both in lung tissue and in

BALF were higher than those of the control rats ($p<0.05$). CUS further enhanced the TGF-β1 levels of the stressed asthmatic rats as compared to the non-stressed asthmatic animals ($p<0.05$). The rats receiving aprepitant showed lower TGF-β1 levels in lung tissue and BALF compared with the stressed asthmatic rats without NK-1 receptor antagonist treatment ($p<0.05$). (Shown in figure 3)

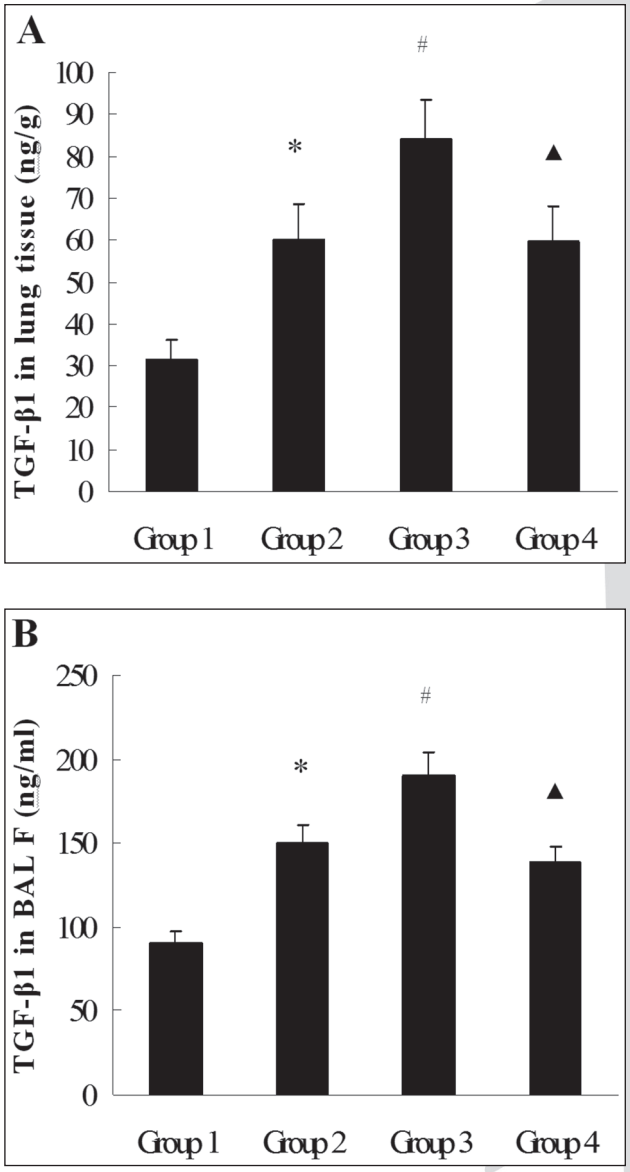



*Figure 3. Levels of TGF-β1 in lung tissue and BALF*

*BAL fluid was collected and lung tissue homogenate was prepared. Levels of TGF-β1 in lung tissue (A) and BALF (B) were determined using ELISA. Data were presented as mean ± SD. * $P<0.05$ vs. Group 1; # $P<0.05$ vs. Group 2; ▲ $P<0.05$ vs. Group 3.*

*Note: Group 1: control group; Group 2: non-stressed asthmatic group; Group 3: stressed asthmatic group; Group 4: treatment group.*

***Inflammatory cytokine assessments***

Levels of the inflammatory cytokines IL-1β and IL-4 were assessed. The OVA sensitization and challenge significantly increased the levels of IL-1β and IL-4 in BALF (both $p<0.05$). Their levels further increased after CUS exposure (both $p<0.05$), while partially abolished by aprepitant (both $p<0.05$). (Shown in figure 4)

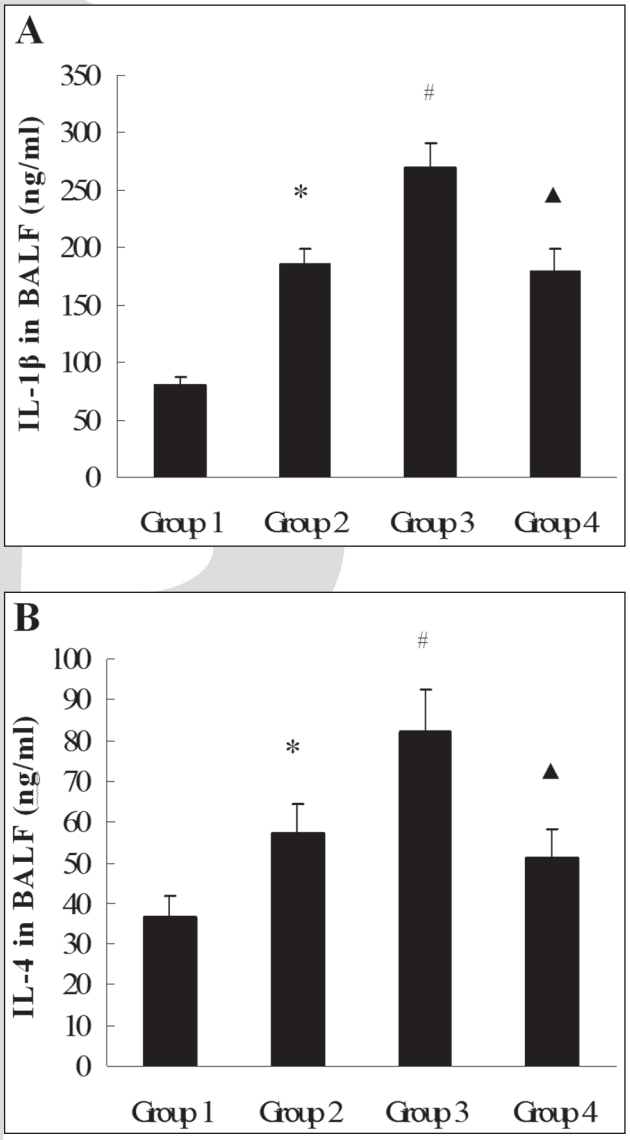



*Figure 4. Levels of cytokines in BALF*

*BAL fluid was collected and levels of IL-1β(A) and IL-4 (B) were determined using ELISA.. Data were presented as mean ± SD. * $P<0.05$ vs. Group 1; # $P<0.05$ vs. Group 2; ▲ $P<0.05$ vs. Group 3.*

*Note: Group 1: control group; Group 2: non-stressed asthmatic group; Group 3: stressed asthmatic group; Group 4: treatment group.*

## Discussion

Our findings provide evidence for the association between CUS and airway inflammation in asthmatic rats. Our data also suggest that substance P might contribute to the influences of chronic stress on asthma.

### *CUS and inflammatory cell infiltration*

Asthma is an inflammatory disease characterized by airway hyperresponsiveness and infiltration of airway mucosa by several types of inflammatory cells (34). Psychological stress occurs at high rates in people with asthma (35, 36), with the lifetime prevalence of depression approaching 50% in patients treated in tertiary care asthma clinics (37). Clinical reports and observational studies, including our previous findings(38), have suggested that psychological factors might exacerbate asthma by increasing the risk of asthma incidence and the symptom severity, leading to more healthcare utilization (36, 39, 40, 41). In an experimental series, an attempt also has been made to study the influence of stress on asthma. A few recent animal studies demonstrated an association between stress and airway inflammation and smooth muscle tonus around the airways in OVA-sensitized animals. But the stress involved in those studies was either acute stress or unimodal repeated stress (9-11), unlike the chronic unpredictable psychological factors that asthmatic patients undergo from daily hassles, sufferings from the disease and treatment failure. In the present study, we exposed the asthmatic rats to chronic unpredictable stress which consisted of unpredictable stressors. Our data indicated that the long-term unpredictable stressors exacerbated the airway inflammation by showing more inflammatory cells including total leukocytes, eosinophils and lymphocytes in the BALF of the stressed asthmatic rats than the non-stressed asthmatic rats. The change of the airway inflammation is similar to the influence of the acute stress on asthma animals reported by Joachim RA (9). But different from our result, only higher number of eosinophils, not including lymphocytes, was found in Joachim RA's study. Our result is also consistent with prior study that reported psychological stress was consistently associated with airway inflammation in asthma patients (42). In that study, the authors also emphasized that effects of acute negative affect must be distinguished from more chronic distress due to daily hassles for asthma patients.

### *CUS and cytokine*

Some inflammatory cytokines play key roles in the initiation and progression of asthma. We assessed the changes of IL-1β and IL-4 which are considered to contribute to the exacerbation of airway inflammation (43, 44). High levels of IL-1β and IL-4 in BALF were found in the non-stressed asthmatic rats. The CUS exposure further increased their levels in the stressed asthmatic rats compared to the non-stressed asthmatic animals. We also measured the level of TGF-β1 which is a cytokine playing an important role in the pathogenesis of the airway remodeling (45). In agreement with findings reported in other studies, we found the asthmatic rats had higher levels of TGF-β1 in lung tissue and BALF. Its levels in the stressed asthmatic rats were further enhanced after the CUS exposure. The result indicated that CUS promoted the pathophysiology of pulmonary inflammation and might contributed to the airway remodeling which could lead to airway hyperresponsiveness.

### *Substance P, a possible way linking chronic unpredictable stress and asthma exacerbation*

One of the main questions raised by this study is the mechanism by which CUS influence airway inflammation and cell damage in asthmatic rats. Substance P, mainly secreted by nerve fibers and immune cells, is involved in some chronic inflammation diseases, such as asthma, and involved in psychological disorders including depression. A few recent studies have provided initial evidence that acute stress or unimodal repeated stress can lead to changes of substance P secretion in OVA-sensitized animals. In this study, we examined the expression of substance P in the lung tissue and BALF after exposure to CUS. Higher levels of substance P were shown in the stressed asthmatic rats compared to the control and the non-stressed asthma rats, indicating that CUS increased the expression of substance P in asthmatic animals. The result was similar to the findings that acute sound stress led to the stimulation of substance P expression in airway-specific neurons in OVA sensitized mice (29). In

contrast to our findings, however, Tohda Y reported that electric shock stress significantly decreased the substance P level in bronchial tissue in biphasic asthma-responsive guinea pigs (28). It is generally accepted that there are potentially several mechanisms by which substance P could lead to greater allergic inflammation and cellular damage. Studies have showed that substance P, being a nerve peptide, could promote activation of inflammatory cells (46). Also, substance P has been shown to increase the production of cytokines which are involved in the pathophysiology of asthma (47). Further, activation of substance P might increase release of vascular endothelial growth factor which is implicated in asthma severity (48, 49). Some other limited available evidence also suggested that inhibition of the NK receptor might decrease airway responsiveness and improve lung function in patients with asthma (50). In our study, we found more severe airway inflammation and enhanced expression of some cytokines together with higher levels of substance P in the stressed asthmatic rats than the non-stressed asthmatic animals. The data indicate that there might be an association between the damage to the lung and substance P induced by CUS.

Further, in order to testify the role of substance P in stress induced asthma exacerbation, we gave highly specific NK-1 receptor antagonist aprepitant to one group of the stressed asthmatic rats. With the application of NK-1 receptor antagonist, the result showed lower number of leukocytes in BALF and lower levels of cytokines (IL-1β, IL-4 and TGF-β1) in stressed asthmatic rats as compared with stressed asthmatic rats without NK-1 receptor antagonist application. The result strongly suggested that higher levels of substance P induced by CUS might be associated with the asthma exacerbation.

Taken together, we conclude that CUS might exacerbate asthma by promoting the airway inflammation and enhancing levels of IL-1β, IL-4 and TGF-β1 in asthmatic rats. CUS could increase levels of substance P in asthmatic rats, and the higher levels of substance P might contribute to the asthma exacerbation. Thus, NK-1 receptor antagonist might have therapeutic potential for the asthmatic patients with psychological disorders such as depression.

However, the activity of hypothalamic-pituitary-adrenal axis, serotonin and T cells (especially Th1, Th2, Th17 and Treg cells) are all involved in the pathophysiology of asthma and psychological stress state. It is necessary to further detect whether CUS influences asthma via those ways and whether substance P has influence on those factors. In addition, further studies are needed to confirm the role of substance P in asthma with psychological stress in human.

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## References

1. *Chengde Li, Shumei Mao, Yuliang Wang, Hongwei Sun. Effects of combination therapy of dexamethasone and fluoxetine on levels of interleukin-1β and interleukin-6 in a rat model of asthma with depressive-like behaviors. HealthMED. 2012, 6(6): 1998-2003.*
2. *Yonas MA, Lange NE, Celedón JC. Psychosocial stress and asthma morbidity. Curr Opin Allergy Clin Immunol. 2012; 12(2): 202-210.*
3. *Ippoliti F, De Santis W, Volterrani A, Canitano N, Frattolillo D, et al. Psychological stress affects response to sublingual immunotherapy in asthmatic children allergic to house dust mite. Pediatr Allergy Immunol. 2006; 17(5): 337-345.*
4. *Chen E, Hanson MD, Paterson LQ, Griffin MJ, Walker HA, Miller GE. Socioeconomic status and inflammatory processes in childhood asthma: the role ofpsychological stress. J Allergy Clin Immunol. 2006; 117(5): 1014-1020.*
5. *Di Marco F, Verga M, Santus P, Giovannelli F, Busatto P, et al. Close correlation between anxiety, depression, and asthma control. Respir Med. 2010; 104(1): 22-28.*
6. *Di Marco F, Santus P, Centanni S. Anxiety and depression in asthma. Curr Opin Pulm Med. 2011; 17(1): 39-44.*
7. *Richardson LP, Russo JE, Lozano P, McCauley E, Katon W. The effect of comorbid anxiety and depressive disorders on health care utilization and costs among adolescents with asthma. Gen Hosp Psychiatry. 2008; 30(5): 398-406.*
8. *Al-Kalemji A, Petersen KD, Sørensen J, Sherson D, Thilsing T, et al. Factors influencing quality of life in asthmatics-a case-control study. Clin Respir J. 2012 Sep 26. doi: 10.1111/crj.12006. [Epub ahead of print]*

9. *Joachim RA, Quarcoo D, Arck PC, Herz U, Renz H, Klapp BF. Stress enhances airway reactivity and airway inflammation in an animal model of allergic bronchial asthma. Psychosom Med. 2003; 65(5): 811-815.*

10. *Okuyama K, Ohwada K, Sakurada S, Sato N, Sora I, Tamura G, Takayanagi M, Ohno I. The distinctive effects of acute and chronic psychological stress on airway inflammation in a murine model of allergic asthma. Allergol Int. 2007; 56(1): 29-35.*

11. *Portela CP, Leick-Maldonado EA, Kasahara DI, Prado CM, Calvo-Tibério IF, Martins MA, Palermo-Neto J. Effects of stress and neuropeptides on airway responses in ovalbumin-sensitized rats. Neuroimmunomodulation. 2007; 14(2): 105- 111.*

12. *Van Lieshout RJ, Bienenstock J, MacQueen GM. A review of candidate pathways underlying the association between asthma and major depressive disorder.Psychosom Med. 2009; 71(2): 187-195.*

13. *Voedisch S, Rochlitzer S, Veres TZ, Spies E, Braun A. Neuropeptides control the dynamic behavior of airway mucosal dendritic cells. PLoS One. 2012; 7(9): e45951. doi: 10.1371/journal.pone.0045951. Epub 2012 Sep 26.*

14. *Xiao L, Wu ZX. Substance p regulates environmental tobacco smoke-enhanced tracheal smooth muscle responsiveness in mice. J Allergy (Cairo). 2012; 2012: 423612. Epub 2012 Aug 13.*

15. *Tibério IF, Leick-Maldonado EA, Miyahara L, Kasahara DI, Spilborghs GM, Martins MA, Saldiva PH. Effects of neurokinins on airway and alveolar eosinophil recruitment. Exp Lung Res. 2003; 29(3): 165-177.*

16. *de Vries A, Engels F, Henricks PA, Leusink-Muis T, McGregor GP, et al. Airway hyper-responsiveness in allergic asthma in guinea-pigs is mediated by nerve growth factor via the induction of substance P: a potential role for trkA. Clin Exp Allergy. 2006; 36(9): 1192-1200.*

17. *Brégeon F, Steinberg JG, Andreotti N, Sabatier JM, Delpierre S, Ravailhe S, Jammes Y. Substance P receptor blockade decreases stretch-induced lung cytokines and lung injury in rats. J Physiol. 2010; 588(Pt 8): 1309-1319.*

18. *Sio SW, Puthia MK, Lu J, Moochhala S, Bhatia M. The neuropeptide substance P is a critical mediator of burn-induced acute lung injury. J Immunol. 2008; 180 (12): 8333-8341.*

19. *Springer J, Groneberg DA, Dinh QT, Quarcoo D, Hamelmann E, et al. Neurokinin-1 receptor activation induces reactive oxygen species and epithelial damage in allergic airwayinflammation. Clin Exp Allergy. 2007; 37(12): 1788-1797.*

20. *Otsuka K, Niimi A, Matsumoto H, Ito I, Yamaguchi M, et al. Plasma substance P levels in patients with persistent cough. Respiration. 2011; 82(5): 431-438.*

21. *Zhang D, Luo XR, Ye XQ, Dong XF. Determination of plasma and sputum substance P content in patients with chronic obstructive pulmonary disease or asthma. Di Yi Jun Yi Da Xue Xue Bao. 2005; 25(10): 1314-1315. [Article in Chinese]*

22. *Yaraee R, Ghazanfari T.Substance P potentiates TGFbeta-1 production in lung epithelial cell lines. Iran J Allergy Asthma Immunol. 2009; 8(1): 19-24.*

23. *Choi JY, Khansaheb M, Joo NS, Krouse ME, Robbins RC, Weill D, Wine JJ. Substance P stimulates human airway submucosal gland secretion mainly via a CFTR-dependent process. J Clin Invest. 2009; 119(5): 1189-1200.*

24. *Roche M, Kerr DM, Hunt SP, Kelly JP. Neurokinin-1 receptor deletion modulates behavioural and neurochemical alterations in an animal model ofdepression. Behav Brain Res. 2012; 228(1): 91-98.*

25. *Ebner K, Singewald N. The role of substance P in stress and anxiety responses. Amino Acids. 2006; 31(3): 251-272.*

26. *Geracioti TD Jr, Carpenter LL, Owens MJ, Baker DG, Ekhator NN, et al. Elevated cerebrospinal fluid substance p concentrations in posttraumatic stress disorder and major depression. Am J Psychiatry. 2006; 163(4): 637-643.*

27. *Herpfer I, Lieb K. Substance P receptor antagonists in psychiatry: rationale for development and therapeutic potential. CNS Drugs. 2005; 19(4): 275-293.*

28. *Tohda Y, Nanbu Y, Tanaka A, Kubo H, Fukuoka M, Nakajima S. Role of substance P in increased airway hypersensitivity following induced stress in a guinea pig asthma model. J Investig Allergol Clin Immunol. 1998; 8(6): 340-345.*

29. *Joachim RA, Cifuentes LB, Sagach V, Quarcoo D, Hagen E, et al. Stress induces substance P in vagal sensory neurons innervating the mouse airways. Clin Exp Allergy. 2006; 36(8): 1001-1010.*

30. *Joachim RA, Sagach V, Quarcoo D, Dinh QT, Arck PC, Klapp BF.Neurokinin-1 receptor mediates stress-exacerbated allergic airway inflammation and airway hyperresponsiveness in mice. Psychosom Med. 2004; 66(4): 564-571.*

31. *Jacob C. Garza, Ming Guo, Wei Zhang, and Xin-Yun Lu. Leptin restores adult hippocampal neurogenesis suppressed by chronic unpredictable stress and reverses glucocorticoid-induced inhibition of GSK3β/β-catenin signaling. Mol Psychiatry. 2012; 17(8): 790–808.*

32. *Cox BM, Alsawah F, McNeill PC, Galloway MP, Perrine SA. Neurochemical, hormonal, and behavioral effects of chronic unpredictable stress in the rat. Behav Brain Res. 2011; 220(1): 106-111.*

33. *Wang W, Sun D, Pan B, Roberts CJ, Sun X, Hillard CJ, Liu QS. Deficiency in endocannabinoid signaling in the nucleus accumbens induced by chronic unpredictable stress. Neuropsychopharmacology. 2010; 35(11): 2249-2261.*

34. *Kim YH, Park CS, Lim DH, Ahn SH, Son BK, Kim JH, Jang TY. Beneficial effect of anti-interleukin-33 on the murine model of allergic inflammation of the lower airway. J Asthma. 2012; 49(7): 738-743.*

35. *Loerbroks A, Herr RM, Subramanian S, Bosch JA. The association of asthma and wheezing with major depressive episodes: an analysis of 245 727 women and men from 57 countries. Int J Epidemiol. 2012; 41(5): 1436-44. doi: 10.1093/ije/dys123. Epub 2012 Aug 9.*

36. *Peters TE, Fritz GK.Psychological considerations of the child with asthma. Child Adolesc Psychiatr Clin N Am. 2010; 19(2): 319-33, ix.*

37. *Nejtek VA, Brown ES, Khan DA, Moore JJ, Van Wagner J, Perantie DC. Prevalence of mood disorders and relationship to asthma severity in patients at an inner-city asthma clinic. Ann Allergy Asthma Immunol. 2001; 87(2): 129-133.*

38. *Sun HW, Wang JP, Wang SZ, Wang YY, Song YP, Yang ZH, Wang LX. Effect of educational and psychological intervention onthe quality of life ofasthmatic patients. Respir Care. 2010; 55(6): 725-728.*

39. *Fagan J, Galea S, Ahern J, Bonner S, Vlahov D. Relationship of self-reported asthma severity and urgent health care utilization to psychological sequelae of the September 11, 2001 terrorist attacks on the World Trade Center among New York City area residents. Psychosom Med. 2003; 65(6): 993-996.*

40. *Theoharides TC, Enakuaa S, Sismanopoulos N, Asadi S, Papadimas EC, Angelidou A, Alysandratos KD. Contribution of stress to asthma worsening through mast cell activation. Ann Allergy Asthma Immunol. 2012; 109(1): 14-19.*

41. *Thomas Ritz. Airway responsiveness to psychological processes in asthma and health. Front Physiol. 2012; 3: 343. Prepublished online 2012 June 6. Published online 2012 September 5.*

42. *Kullowatz A, Rosenfield D, Dahme B, Magnussen H, Kanniess F, Ritz T. Stress effects on lung function in asthma are mediated by changes in airway inflammation. Psychosom Med. 2008; 70(4): 468-475.*

43. *Zhang Y, Xu CB, Cardell LO. Long-term exposure to IL-1beta enhances Toll-IL-1 receptor-mediated inflammatory signaling in murine airway hyperresponsiveness. Eur Cytokine Netw. 2009; 20(3): 148-156.*

44. *Siegle JS, Hansbro N, Dong C, Angkasekwinai P, Foster PS, Kumar RK. Blocking induction of T helper type 2 responses prevents development of disease in a model of childhood asthma. Clin Exp Immunol. 2011; 165(1): 19-28. doi: 10.1111/j.1365-2249.2011.04392.x. Epub 2011 Apr 19.*

45. *Yum HY, Cho JY, Miller M, Broide DH. Allergen-induced coexpression of bFGF and TGF-β1 by macrophages in a mouse model of airway remodeling: bFGF induces macrophage TGF-β1 expression in vitro. Int Arch Allergy Immunol. 2011; 155(1): 12- 22.*

46. *Ramalho R, Almeida J, Beltrão M, Pirraco A, Costa R, et al. Neurogenic inflammation in allergen-challenged obese mice: A missing link in the obesity-asthmaassociation? Exp Lung Res. 2012; 38(6): 316-324. doi: 10.3109/01902148.2012.699589.*

47. *Shi X, Wang L, Li X, Sahbaie P, Kingery WS, Clark JD. Neuropeptides contribute to peripheral nociceptive sensitization by regulating interleukin-1β production in keratinocytes. Anesth Analg. 2011; 113(1): 175-183.*

48. *Castellani ML, Galzio RJ, Felaco P, Tripodi D, Toniato E, et al. VEGF, substance P and stress, new aspects: a revisited study. J Biol Regul Homeost Agents. 2010; 24(3): 229-237.*

49. *Theoharides TC, Zhang B, Kempuraj D, Tagen M, Vasiadi M, et al. IL-33 augments substance P-induced VEGF secretion from human mast cells and is increased in psoriatic skin. Proc Natl Acad Sci U S A. 2010; 107(9): 4448-4453.*

50. *Ramalho R, Soares R, Couto N, Moreira A. Tachykinin receptors antagonism for asthma: a systematic review. BMC Pulm Med. 2011; 11: 41.*



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# Recovery Effect of Basil on ovarian tissue artery Hyperemia after exposed with electromagnetic field

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**Abstract**

**Background:** Medicinal use of basil, Ocimum basilicum, dates back to ancient times in Iran, China, and India.

**Objectives:** This herb has been used since ancient times as a medicine and food and it is known that the antioxidant effect of O. basilicum is beneficial to protect tissue and decreasing carcinogenic effect of EMF, so it was hypothesized that this herb might also provide protection ovarian tissue from reactive oxygen spaces (ROS).

**Materials and Methods:** Female wistar rats (n = 40) were allocated to four groups, a control group (n = 10) and three treatment groups (n = 30). The first treatment group received O. basilicum extract (1.5 g/kg body weight), the second extract group received O. basilicum extract (1.5 g/kg body weight) and EMF exposure at 50 Hz for 40 consecutive days, whilst the third group received only EMF exposure for 40 consecutive days. At the conclusion of the test period rat ovary tissues were removed from all group members. Ovary tissue preparation was performed and analyzed for apoptosis.

**Results:** There was a significant increase in apoptosis in EMF group when compared with other groups ($P<0.05$). EMF has negative effect on ovary histology in rats by increasing ROS. However, these side effects are less seen in the EMF group that received O. basilicum extract. **Conclusion**: Therefore, it is recommended that usage of O. basilicum extract in modern country has fewer side effects of industrial as one of female cancer (ovary cancer) problems.

**Key words:** Apoptosis, EMF, Ocimum basilicum, ovary.

## Introduction

The antioxidant capacity of phenolic compounds, flavonoids, and foods rich in these compounds, has been repeatedly demonstrated in various in vitro and in vivo systems (1). Ocimum basilicum (Basil) is an annual herb of the Lamiaceae family, which is widely cultivated in Asia as a nourishing food and herbal medicine. O. basilicum is widely used in folk medicine to treat a wide range of diseases. For example, the aerial part of O. basilicum is traditionally used as an antispasmodic, aromatic, digestive, carminative, stomachic, and tonic agent. O. basilicum has also been used externally for the topical treatment of acne, insect stings, snake bites, and skin infections (2,3).An electromagnetic field (EMF or EM field) is a physical field produced by electrically charged objects. EMFs affect the behavior of charged objects in the vicinity of the field. The EMF extends indefinitely through space and determines electromagnetic interaction (4,5). EMF is one of the four fundamental forces of nature; the others are gravitation, the weak interaction, and the strong interaction. The EMF can be viewed as a combination of an electric field and a magnetic field. The increased use of power lines and modern electrical devices is of concern as a public health hazard, and chronic exposure to EMF has attracted considerable attention. Exposure to EMF adversely affects spermatogenesis by the Sertoli and Leydig cells(6). Magnetic fields of 50 Hz also induce cytotoxic and cytostatic changes in the differentiating spermatogonia of mice (7). Furthermore, the study about effects of EMF in female rats showed: During the development of ovarian follicle in human, recruitment of a cohort of follicles occur, with only one destined to ovulate. In 2007 solimany rad and roshangar conformed previous researchs that done

by Gougeon and present the remainder of the cohort undergoes atresia by uncertain stimuli and mechanisms. It appears that growth factors and related peptides may are involved in this process (8-10). It is suggested that follicular atresia in the ovary results from apoptosis (11), The present study was designed to investigate about protective effects O. basilicum as anti-oxidanton ELF-EMF effects on ovary cells apoptosis.

## Material and Methods

A total of 40 female Wistar rats were maintained for use in this study. Rats were housed together (10 per cage) and fed on a compact diet in the form of granules and water. The diet contained all the essential ingredients, including, vitamins and minerals. The environmental conditions (temperature and humidity) in all the animal holding areas were continuously monitored. Temperature was maintained in the range of 23 °C and humidity was maintained at 35–60%. Light was provided on a 12 h light/dark cycle from 0700 h to 1900 h. All animals were treated in accordance to the Principles of Laboratory Animal Care [NIH]. The experimental protocol was approved by the Animal Ethics Committee in accordance with the guide for the care and use of laboratory animals prepared by Tabriz University of Medical Sciences. Rats were allocated to four groups, a control group (n = 10) and three treatment groups (n = 30). The first treatment group received O. basilicum extract (1.5 g/kg body weight), the second extract group received O. basilicum extract (1.5 g/kg body weight) and ELF-EMF exposure at 50 Hz for 40 consecutive days, while the third group received only ELF-EMF exposure for 40 consecutive days. Animals were maintained under standard conditions.

### *ELF-EMF-producing system*

The equipment was based on the Helmholtz coil, which operated following Fleming's right hand rule. The equipment produced an alternating current of 50 Hz, which created an EMF of 80 G. The intensity of the EMF was controlled using a transformer. The equipment had two main parts. In the first part, there were two copper coils placed one above the other and separated by a distance of 50 cm. A cylindrical wooden vessel was placed between the coils (the exposure area), the interior of which contained a chamber for holding the caged experimental animals. The second part was the transformer, which controlled the input and output voltage using a voltmeter and the current with an ampere meter. A fan was used as required, to prevent increases in temperature inside the chamber. Four cages at a time were placed within the chamber, with or ten rats per cage.

### *Surgical procedure*

On day 40, a sodium pentobarbital solution (40 mg/kg) was administered intra-peritoneally as an anesthetic, and the peritoneal cavity was opened with a lower transverse abdominal incision. The ovary tissues immediately were removed from the control and experimental groups. The weight of the testes for each group member was recorded. Animals were then decapitated between 10:00 h and 12:00 h.At the end of 4 weeks of treatment, ovary was dissected from each rat ,24 h after the last administration Then tissue preparation was performed to investigate ,artery hyperemia and ovarian cells apoptosis by TUNEL method.

### *TUNEL analysis of apoptosis*

The in-situ DNA fragmentation was visualized by TUNEL method (Khaki et al.,2008). Briefly, dewaxed tissue sections were predigested with 20 mg/ml proteinase K for 20 min and incubated in phosphate buffered saline solution (PBS) containing 3 % H2O2 for 10 min to block the endogenous peroxidase activity. The ovarian sections were incubated with the TUNEL reaction mixture, fluorescein-dUTP (in situ Cell Death Detection, POD kit, Roche, Germany), for 60 min at 37°C. The slides were then rinsed three times with PBS and incubated with secondary anti-fluorescein-POD-conjugate for 30 min. After washing three times in PBS, diaminobenzidine- $H_2O_2$ (DAB, Roche, Germany) chromogenic reaction was added on sections and counterstained with hematoxylin. As a control for method specificity, the step using the TUNEL reaction mixture was omitted in negative control serial sections, and nucleotide mixture in reaction buffer was used instead. Apoptotic cells were quantified by counting the number of TUNEL stained nuclei per ovarian cross section. 100 cross sections of per specimen were assessed and

the mean number of TUNEL positive dark brown cells per each cross- section was calculated.

***Extract preparation***

Fresh basil was prepared from local shopping in Tabriz. Superfluous materials were rub off and were drained. Dried plants were steeped in methanol (90°) Merck company, then extract were exploited in vacuum condition. Prepared extract were dried and used in maximum two days.

***Measurement of Serum Total Antioxidant capacity (TAC)***

TAC was measured in serum by means of a commercial kit (Randox Co-England). The assay is based on the incubation of 2, 2'-azino-di-(3-ethylbenzthiazoline sulphonate) (ABTS) with a peroxidase (methmyoglobin) and hydrogen peroxide to produce the radical cation ABTS+, which has a relatively stable blue-green color, measured at 600 nm. The suppression of the color is compared with that of the Trolox, which is widely used as a traditional standard for TAS measurement assays, and the assay results are expressed as Trolox equivalent (mmol/L).

**Results**

Our finding showed, ovarian apoptotic granulosa cells percentage significantly was decreased following administration of O. basilicum extract (1.5 g/kg body weight) in compared to the control group($P<0.05$). Exposed to 50 Hz of ELF-EMF caused a significant increase in the apoptotic granulosa cells percentagees. When 50 Hz of ELF-EMF was administrated together with O. basilicum extract (1.5 g/kg body weight).apoptotic granulosa cells percent was significantly decreased ($p < 0.05$) in granulosa cells ,respectively Artery hyperemia significantly decreased($p < 0.05$) from. These results Indicating the protective effect of O. basilicum against ELF-EMF- induced granulosa cells apoptosis, other results showed percentage of large antral follicle and Ovary weight's were significantly decreased in groups with exposed to 50 Hz of ELF-EMF($p < 0.05$) but following administration of O. basilicum extract (1.5 g/kg body weight) can re uptake this decreasing, in other hand large antral follicle in group that receiving O. basilicum was significantly increased when compared to other study groups . artery hyperemia was significantly increased in groups with exposed to 50 Hz of ELF-EMF($p < 0.05$) and O. basilicum (1.5 g/kg body weight) can modify this harmful effect of ELF-EMF (Table 1).

**Discussion**

In fertility is one of the major problems in matchs life, about 35 percent of infertility is regard to woman (12). The importance of many of these factors is not yet clearly understood. A better understanding of underlying mechanisms in fertility and better study results clarifying the effectiveness of nutritional and biochemical factors are important to improve diagnosis and treatment. Smart choices for better foods might prevent body from many diseases (13,14). It has been suggested that lifetime of free radicals depended to electric and magnetic fields at environmental levels and it may extend the and result in DNA damage (6,15). EMF by affecting biochemical reactions cause to product unpaired electron such as a superoxide ion, nitrogen oxide and hydroxyl radical, ion channels,

*Table 1. Granulosa cells Apoptosis, Large antral Follicle & Artery hyperemia percentage, TAC and ovary weights of rats witch exposed to EMF and O. basilicum Extract.*

| O. basilicum + (EMF) | O. basilicum (1.5 g/kg body weight) | EMF (50Hz) | control | groups |
|---|---|---|---|---|
| 11.05 ± 0.05✶ | 3.45 ± 0.01 | 15.33 ± 0.05✶ | 4.01 ± 0.03 | Granulosa apoptotic cell (%) |
| 9.05 ± 0.05✶ | 13.25 ± 0.05✶ | 05.01 ± 0.05✶ | 10.05 ± 0.05 | Large antral Follicle (%) |
| 1.40 ± 0.371 | 1.57 ± 0.73 | 1.00 ± 0.01✶ | 1.50 ± 0.05 | Ovary weight's(Gram) |
| 1.5 ± 0.01✶ | 0.04 ± 0.03 | 3.90 ± 0.05✶ | 0.05 ± 0.01 | Artery hyperemia (%) |
| 1.1 ± 0.05 | 2.01 ± 0.05✶ | 0.75 ± 0.05✶ | 1.8 ± 0.05 | TAC(mmol/ml) |

*Data are presented as mean ± SE.*

✶ *Significantly different at $P < 0.05$ level (compared with the control group).*

synthesis of macromolecules could have a harmful effect on cellular metaboli sm and cause to cells damages (15-18).Plants and natural products are extensively used in several traditional systems of medicine, so screening these products for radio-protective compounds has several advantages, because they are usually considered non-toxic and are widely accepted by humans. Many natural antioxidants, whether consumed before or after radiation exposure, can confer some level of radioprotection. In addition to beneficial effects accrued from established antioxidants, such as, vitamin C and E, and their derivatives, vitamin A, beta carotene, curcumin, Allium cepa, quercetin, caffeine, chlorogenic acid, ellagic acid, and bixin, protection is also conferred by several novel molecules, including, flavonoids, eppigallocatechin, and other polyphenols (19-22). the findings of the present study indicate that ELF-EMF cause to apoptosis of granulosa cells and this is responsible for initiation of follicular atresia and degeneration. In this study EMF could significantly increasing arteriole hyperemia and decreasing in ovary weights and number of large antral follicles, so it seems EMF as an environmental factor, could disturb folliculogenesis by inducing apoptosis in granulosa cells. Regarding the universal increase of infertility rate (23), further studies are needed to clarify the relation between EMF exposure and infertility in mammals.EMF-induces as one apoptosis agent in alterations in the oocyte and could be considered as a pre apoptotic status of oocyte. Irregular morphology of nucleus could be an indication of changes in nuclear skeleton. A change in cytoskeletal proteins and degradation of nuclear lamin is considered as a trigger of apoptosis cascade (24).Although the mechanisms underlying follicular reversible or irreversible cell damage are not well known at this time, DNA damage, which can be initiated by oxidative stress such as free radicals role, it has been proposed as a possible mechanism that leads to the activation of the apoptotic cascade in atretic follicles (25). In support of this idea; it is shown that EMf has a pro-oxidant effect (26) and it is proposed that the effect of EMF is mediated by production of free radicals (22). According to Peluso et al in 1977, graulosa cells in atretic follicles undergo nuclear condensation and cytoplasmic blebbing. Based on roshangar & solimany rad research in 2007 type of changes occurred in EMF-exposed granulosa cells and followed by appearance of apoptotic bodies, a characteristic of late apoptosis. Our findings well agrees with pro-oxidant effect of EMF (26) and that, oxidants are well known apoptosis-inducing factors (27-29). A disturbance in the pro-oxidant/antioxidant system has been defined oxidative stress. Reactive oxygen species (ROS) are very reactive molecules ranked as free radicals owing to the presence of one unpaired electron such as a superoxide ion, nitrogen oxide and hydroxyl radical, administration of this extract able to co-unterbalance the negative effect of ELF-EMF on ovary tissue. previous study showed that 2 hours of 60 Hz EMF exposure immediately altered the metabolism of free radicals, decreased SOD activity in plasma, decreased GSH content in the heart and kidney, but did not induce immediate lipid peroxidation(21), EMF is able to generate destructive reactive oxygen species including superoxide, hydrogen peroxide and hydroxyl radical and frequently used to produce oxidative and necrotic damages(20). The role of EMF in the induction of apoptosis and oxidative damage has also been reported. This could be indicative of free radical scavengering properties of Ocimum basilicum (30). The results of other study showed the anti-oxidant ability of Ocimum basilicum in the enhancement of protective effects of EMF exposures rats resulting from decrease of apoptosis in testis and vein congestions decreasing. This study demonstrated that the administration of Ocimum basilicum can overcome reproductive toxicity of EMF effects. In conclusion This natural extract (Ocimum basilicum) as an anti-oxidant can protect ovary tissue and follicles and it also able to reduce apoptosis in ovary tissue.

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## References


1. *Alexandopoulou I, Komaitis M, Kapsokefakou M. Effects of iron, ascorbate, meat, and casein on the antioxidant capacity of green tea under conditions of in vitro digestion. Food. Chem. 2006; 94(3): 359-65.*

2. *Supawan B, Chanida P, Nijsiri R. Chemical compositions and antioxidative activities of essential oils from four ocimum species endemic to Thailand. J .Health Res. 2007; 21(3): 201-6.*

3. *Khaki A, Fathiazad F, Nouri M, Khaki AA. Effect of Ocimum basilicum on apoptosis in testis of rats after exposure to electromagnetic field. AJPP. 2011; 5(12): 1534–1537*

4. *Schüz J, Lagorio S, Bersani F. Electromagnetic fields and epidemiology: an overview inspired by the fourth course at the International School of Bioelectromagnetics. Bioelectromagnetics. 2009; 30(7): 511-24.*

5. *Emre M, Cetiner S, Zencir S, Unlukurt I, Kahraman I, Topcu Z. Oxidative stress and apoptosis in relation to exposure to magnetic field. Cell. Biochem. Biophys. 2011; 59(2): 71-7.*

6. *Michael SL, Pumford NR, Mayeux PR, Niesman MR, Hinson JA. Pretreatment of mice with macrophage inactivators decreases acetaminophen hepatotoxicity and the formation of reactive oxygen and nitrogen species. Hepatology, 1999; 30: 186–195.*

7. *Khaki AA, Zarrintan S, Khaki A, Zahedi A. The effects of electromagnetic field on the microstructure of seminal vesicles in rat: a light and transmission electron microscope study. Pak. J .Biol Sci. 2008; 11(5): 692-701.*

8. *Roychoudhury S, Jedlicka J, Parkanyi V, Rafay J, Ondruska L, Massanyi P, Bulla J. Influence of a 50 hz extra low frequency electromagnetic field on spermatozoa motility and fertilization rates in rabbits. J. Environ Sci Health A Tox Hazard Subst Environ Eng. 2009; 44(10): 1041-7.*

9. *Wei YC, Giudice L. Programmed cell death in human ovary is a function of follicle and corpus luteum status. Clin. Endocrinol., 1997; 82: 3148-3155.*

10. *Soleimani Rad J, Roshangar L, Karimi K. The effect of electromagnetic field on folliculogenesis in ovary. J. Iran. Anatom. Sci., 2003; 1: 47-5 .*

11. *Williams GT, Smith CA. Molecular regulation of apoptosis: Genetic controls on cell death. Cell, 1993; 74: 777-779.*

12. *Mosher WD, Pratt WF. Fecundity and infertility in the United States: incidence and trends. J. Fertil Steril. 1991; 56(2): 192 - 3.*

13. *Reddy PS, Pushpalatha T, Reddy PS. Reduction of spermatogenesis and steroidogenesis in mice after fentin and fenbutatin administration. Toxicol. Lett. 2006; 166: 53 - 9.*

14. *Suryavathi V, Sharma S, Sharma S, Saxena P, Pandey S, et al. Acute toxicity of textile dye wastewaters (untreated and treated) of Sanganer on male reproductive systems of albino rats and mice. Reprod. Toxicol. 2005; 19: 547-56.*

15. *Lai H, Singh NP. Magnetic-Field-Induced DNA Strand Breaks in Brain Cells of the Rat. Environmental Health Perspectives, 2004; 112(6): 687-694*

16. *Levin LA. Retinal ganglion cells and neuroprotection for glaucoma. Surv Ophthalmol 2003; 48: S21–S24.*

17. *Khaki A, Imani SAM, Golzar SF. Effects of rosmarinic acid on male sex hormones (testosterone-FSH-LH) and testis tissue apoptosis after exposure to electromagnetic field (EMF) in rats. AJPP. 2012; 6(4): 248 - 252 .*

18. *Khaki A, Ghaffari Novin M, Khak AA, Fathiazad F, Khabiri M, Hossinchi J. Ultra Structural Study of Gentamicin and Ofloxacin Effect on Testis Tissue in Rats: light and Transmission Electron Microscopy. AJPP. 2009; 3(4): 105 - 9.*

19. *Kirtikar KR, Basu BD "Indian Medicinal Plants", 2nd ed., Periodical Export, New Delhi. 1991.*

20. *Khaki A, Heidari M, Ghaffari Novin M, Khaki AA. Adverse effects of ciprofloxacin on testis apoptosis and sperm parameters in rats. Iranian J. Reproductive Medicine. 2008; 6(2): 14 – 20.*

21. *Khaki A, Fathiazad F, Nouri M, Khaki AA, Chelar C, et al. The Effects of Ginger on Spermatogenesis and Sperm parameters of Rat. Iranian J. Reproductive Medicine. 2009; 7(1): 7 - 12.*

22. *Khaki A, Fathiazad F, Nouri M, Khaki AA, Jabbari Khamenhi H, Hammadeh M. Evaluation of Androgenic Activity of Allium cepa on Spermatogenesis in Rat. Folia Morphologica. 2009; 68(1): 45 - 51.*

23. *Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Hum Reprod; 2007; 22: 1506-1512.*

24. *Andrew Vicki D, Bertram F, Hill D, Mansow S. Apoptosis and cell proliferation 2nd Edn. Boehringer Mannheim. 1998.*

25. *McLauchlan KA. A possible mechanism for the effect of electromagnetic field on biological cells. Sci.Thechnol., 1980; 84: 46-48*

26. *Regoli F, Gorbi S, Machella N, Tedesco S, Benedetti M, et al. Pro-oxidant effects of Extremely low frequency electromagnetic fields in the land snail Helix aspersa. Free Radica. Biol. Med., 2005; 39: 1620-1628.*

27. *Chandra J, Samali A, Orrenius S. Triggering and modulation of apoptosis by oxidative stress. Free Radio Biol. Med., 2000; 29: 323-330.*

28. *Jiang S, Moriarty SE, Grossniklaus H. Increased oxidant-induced apoptosis in cultured nondividing human retinal pigment epithelial cells. Invest. Ophthalmol. Vis. Sci., 2002; 43: 2546-2553.*

29. *Jiang S, Moriarty SE, Drr M, Cai J, Stemberg P, Jones DP. Oxidant-induced apoptosis in human retinal pigment epithelial cells: Dependence on extracellular redox state. Invest Ophthalmol. Vis. Sci., 2005; 46: 1054-1061.*

30. *Polat A, Parlakpinar H, Tasdemir S, Colak C, Vardi N, et al. Acet A. Protective role of aminoguanidine on gentamicin-induced acute renal failure in rats. Acta Histochemica. 2006; 108: 365 - 71.*

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# Therapeutic effect and safety of internal fixation via anterior lateral approach in treatment of close vertical plane pilon fracture

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**Abstract**

**Objective:** To evaluate the therapeutic effect and safety of internal fixation via anterior lateral approach in treatment of close vertical plane Pilon fracture.

**Methods:** From Jan. 2008 to Jun. 2012, 34 patients (19 male and 16 female, 19-64 years old) with close vertical plane Pilon fracture were treated with internal fixation with locking plate via anterior lateral approach. According to Ruedi-Allgowe typing method, 9 patients were type I, 17 patients were type II and 8 patients were type III.

**Results:** All the patients were followed up for 6 to 24 months (12±4.7 months on average). In all the 34 patients, bone union was achieved in 32 patients, and the healing time ranged from 3 months to 9 months (5.2±3.7 months on average). The therapeutic efficacy was excellent in 16 patients, good in 15 patients, acceptable in 2 patients and bad in 1 patient. The good rate of therapeutic effect was 91.2%, and there was no looseness and falling out in internal fixations, no bone infection and other complications.

**Conclusion:** The strategy of internal fixation via anterior lateral approach in treatment of close vertical plane Pilon fracture is safe and effective. It can provide useful information for clinical application.

**Key words:** Pilon fracture, internal fixation, therapeutic effect and safety.

## Introduction

Pilon fracture, as one of the most challenging fractures, is a kind of distal tibial fracture involving tibiotalar articular surface. Severely comminuted distal tibial articular surface, bone defects, distal cancellous bone compression, and often complicated with lower fibula fractures and severe soft tissue contusion are the clinical manifestation of Pilon fracture. The fixing way of Pilon fracture has always been controversial. Here we reported 34 patients with close vertical plane Pilon fracture admitted in Orthopedics Department of our hospital from January 2008 to June 2012. All the patients were treated by internal fixation via anterior lateral approach, of which efficacy was also confirmed.

## Material and Methods

### *General information*

Thirty-four patients with close vertical plane Pilon fracture admitted in Orthopedics Department of our hospital from January 2008 to June 2012 were enrolled, including 19 males and 15 females with 19 to 64 years old (47 ± 7.9 years on average). The causes of fractures comprised of 12 cases of falling injury, 11 cases of traffic accidents, 7 cases of injury by crashing objects, and 4 cases of ground falls, and there were 18 cases of left side fracture and 16 cases of right side fracture. According to Ruedi-Allgowe typing method, 9 cases were type I, 17 cases were type II and 8 cases were type III. All the patients were taken post-anterior and lateral X-ray films on ankle and lower tibia and fibula, and then emergency surgery was carried out or basic treatment of calcaneal traction, cast immobilization and dehydration for detumescence were performed before elective surgery [1].

### *Operation procedures*

Combined spinal epidural anesthesia or general anesthesia was applied. The patients were in a supine position after anesthesia success, and routine disinfection shop towels and tourniquets were used. Fibula internal fixation was firstly performed in patients with fibular fracture, in order to restore fibular

length to determine the length of tibia and to avoid affecting the stability of ankle joint. A longitudinal incision about 15 cm was made along with the interior edge of distal fibula at the anterolateral lower leg. Cutting the skin, subcutaneous tissues, deep fascia, exposing superior extensor support ligament and cutting, isolating the third peroneal muscle for internal traction of its tendons and extensor digitorum longus tendons, cutting the periosteum and joint capsule, dissecting subperiosteal to expose anterolateral distal tibia, the fracture of tibial articular surface as well as the leading edge of tibiofibular joint, and bluntly separating to the outside and inner rear side of the ankle [2]. The anterior tibial artery and peroneal nerve should be protected during the separation process above. The bulk fracture fragments were reset to try to restore their anatomical location using talus as a reference, or reset the tibial articular surface according to chaput nodule. Temporary fixation was performed with Kirschner after reset, and appropriate hollow screws were applied for fixation after satisfactory reset confirmed by bedside X-ray. Bone graft was used on bone defects to restore the flat articular surface. Finally, locking plate was used for internal fixation to repair joint capsule and ligaments, suturing and placing a suction drainage tube, bandaging with a sterile dressing and fixing with plaster.

***Postoperative treatment***

The postoperative treatment comprised of postoperative immobilization, routine use of antibiotics, asking the patients to lift the injured leg, giving repercussive drugs, asking appropriate toe activities at 3 to 4 days after operation, and plaster fixation for 2 to 3 weeks. The patients were guided for ankle functional exercise after wound healing and weight-bearing should be avoided in early stage. Post-anterior lateral X-ray films were periodically reviewed, and continuity callus suggested weight-bearing exercise. Appropriate to increase of weight was allowed until the X-ray showed the fracture healed well.

***Efficacy evaluation standards***

On the basis of post-anterior lateral X-ray films, the evaluation was carried out by using the ankle symptoms and function scores developed by Mazur et al [3]. Excellent: ankle non-swelling, free movement and normal gait with score more than 92 points; Good: slight ankle joint pain, activity equivalent to three-quarters of the normal, and normal gait with score from 87 to 92 points; Acceptable: ankle pain, activity equivalent to half of the normal, normal gait, and need to take anti-inflammatory drugs with score from 65 to 86 points; Poor: ankle swelling and pain, activity equivalent to half of the normal, and limp with score less than 65 points.

## Results

All the patients were followed up for 6 to 24 months (mean 15 ± 4.7 months). 32 patients showed bone healing, and the healing time was 3 to 9 months (mean 5.2 ± 3.7 months). The callus was not observed after 6 months in 2 patients and the fracture fragment absorption was apparent, considering nonunion fracture. After the injection of autologous red bone marrow, the bone healing was observed at the 9 months and 11 months after operation, respectively. According to the efficacy evaluation standards above, the efficacy of the 34 patients was as follows: excellent in 16 patients, good in 15 patients, acceptable in 2 patients, and poor in 1 patient with a good rate of 91.2%. The 1 patient with poor efficacy was due to severe articular comminuted fracture and articular surface still not smooth after repair, which lead to joint pain, obvious limp, and ankle joint degeneration shown by X-ray films. All patients had no loosening or shedding of internal fixation, and no bone infection or other complications occurred. The typical case was shown in Figure 1 and Figure 2.

## Discussions

Pilon fractures is a kind of distal tibial 1/3 fractures involving tibiotalar articular surface with the features of severely comminuted distal tibial articular surface, bone defects and distal cancellous bone compression, which make clinical treatment more difficult. The treatment of Pilon fracture has always been controversial, mostly focusing on different perspectives on the choice of fixation and operation time.

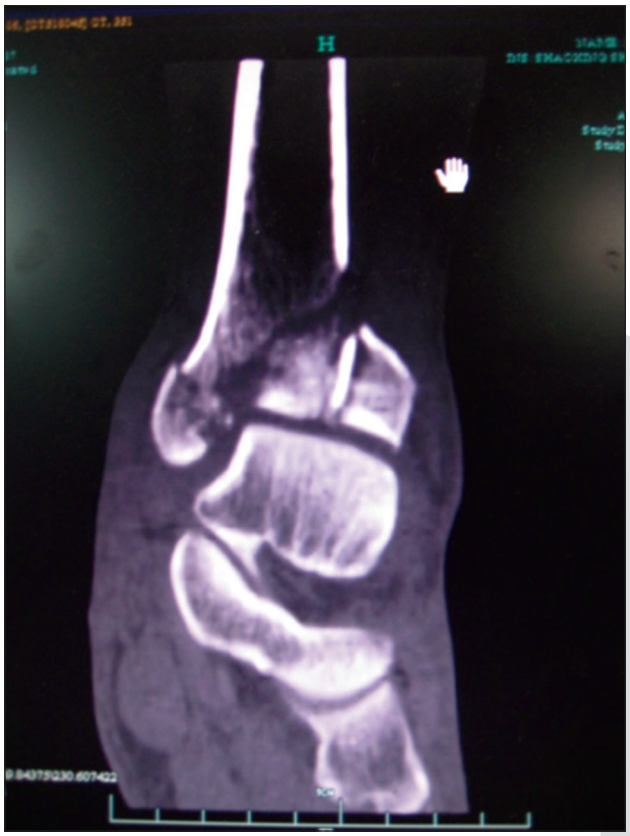

*Figure 1.*

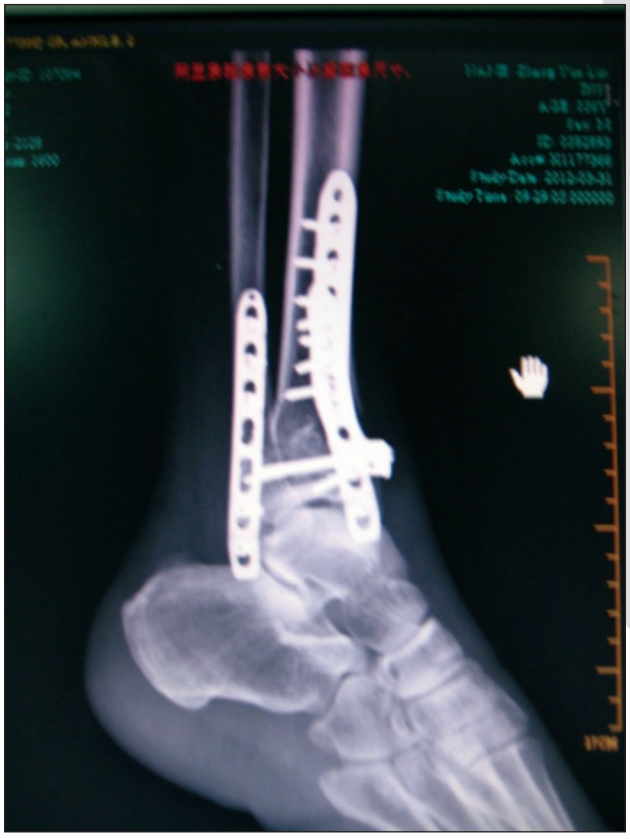

*Figure 2.*

***Surgical timing***

Pilon fractures are often complicated with varying degrees of soft tissue injury, and surgical efficacy is dependent on the control of local complications to some extent [4]. Therefore, if patients with severe soft tissue injury, the treatment for which should be prior to fracture surgery, and traction, manual restoration, elevation of injured leg to make the ankle in a neutral position, restoring limb length, and alleviating limb swelling should be carried out. After the local conditions improve, then open restoration was performed [5]. Avoiding surgery in the acute phase of soft tissue injury is helpful to reduce postoperative soft tissue complications, bone infection, bone nonunion and other complications, and provides a good biological environment for bone healing after surgery. For open fractures, early debridement and external fixation are generally recommended, which can delay the reconstruction of bone and soft tissues [6].

***Surgical approach***

The choice of surgical approach determines the exposure way and internal fixation placement of fracture [7]. Some studies believe that the vertical plane Pilon fractures is associated with high-energy mechanism of injury [8], and the high energy injury caused Pilon fractures belong to the AO classification type C3 fractures [2]. In clinical practice, the fracture pieces of this type of fracture often locate in the anterolateral tibial epiphysis, and anterolateral surgical approach is helpful to reset fractures and placing fixtures. Some literatures reported anterolateral surgical approach for severe Pilon fractures obtained good efficacy [2, 9-11]. In this study, we adopted the anterolateral surgery approach, the range of operation field was well exposed, and anterolateral L-shaped locking plate was also easily placed. All the 34 patients achieved satisfactory efficacy.

***Fixation***

The choice of fixation ways has been controversial [12]. In recent years, the clinical application of internal fixation or limited internal fixation achieves good efficacy [13-14]. It is generally believed that as long as the articular surface fractures are not severe, open reduction and internal fixation is recommended. In this study, we use locking plates for internal

fixation. 32 patients achieved bone healing with a therapeutic good rate of 91.2%, and no loosening and shedding of internal fixation or bone infections occurred. We believe that locking plate fixation has several advantages, including: 1. The locking plate is designed according to the anatomical structure of the fracture site, and do not need precise shaping to facilitate placement in surgery; 2. The screws are threaded so that it can work closely with the steel plate, and the angle between the screws and steel plate may play a stabilizing role. It is suitable for patients with comminuted fracture and osteoporosis, and functional exercise can be carried out in early postoperative period; 3. The steel plate neither affects local soft tissue injury, nor generates pressure on the bone. No periosteal stripping procedure and less bleeding maximize the protection of the blood supply of the fracture site, which is helpful for fracture healing; 4. Locking plate has pressure screw holes and suture screw holes, and lag screws can be used to restore the fracture and fracture fragments. The temporarily fixation by steel needle is available after restoration, and is in accordance with biomechanical principles [15].

Taken together, the clinical treatment of Pilon fracture is complicated and specific. Comprehensive evaluation of patient's condition is necessary for the development of rational therapeutic strategies. We used anterolateral surgical approach and locking plate fixation in treatment of 34 patients with close vertical plane Pilon fracture, and 32 patients achieved bone healing with therapeutic rate of 91.2%. No loosening and shedding of internal fixation, bone infection and other complications occur. The treatment strategy is a safe and effective way for clinical reference.

## References


1. *Chen Yu, Yang Huilin, Wang Genlin, et al.. Treatment of Ruedi-Allgower II, III Pilon fractures through anterior center limited incison [J]. Chinese Journal of Orthopaedic Trauma, 2012; 14(9): 755-758.*
2. *Guo Ningguo, Feng Jichuan, Liu Tao, et al.. Treatment of severe Pilon fractures through anterolateral surgical approach [J]. The Journal of Traditional Chinese Orthopedics and Traumatology, 2012; 24(8): 43-44.*
3. *Shuang Z,Pengfei L, Jin L. Clinical effect of the staged delayed therapy of severe pilon fracture [J]. Chinese Journal of Coal Industry Medicine, 2012; 2: 010.*
4. *Calori G, Tagliabue L, Mazza E, et al.. Tibial pilon fractures: Which method of treatment?[J]. Injury, 2010; 41(11): 1183-1190.*
5. *Cronier P, Steiger V, Rammelt S. Early open reduction and internal fixation of Pilon fractures[J]. Fuß & Sprunggelenk, 2012.*
6. *Zhang Kefei. Choice of surgical timing of open restoration and internal fixation of tibial Pilon fractures [J]. Clinical Journal of Medical Officer, 2008; 36(4): 564-565.*
7. *Zhang Jian, Wang Manyi, Gong Xiaofeng, et al.. Posterolateral approach intreatment of posterior Pilon fractures [J]. Chinese Journal of Joint Surgery (Electronic Edition), 2011; 5(4): 16-19.*
8. *Topliss C, Jackson M, Atkins R. Anatomy of pilon fractures of the distal tibia[J]. Journal of Bone & Joint Surgery, British Volume, 2005; 87(5): 692-697.*
9. *Yang Hua, Lei Guanghua, Li Kanghua, et al.. Distal tibia anterolateral anatomical plate internal fixation intreatment of Pilon fractures [J]. Journal of Clinical Research, 2008; 25(004): 633-635.*
10. *Shi Dehai, Lu Huading, Li Donghui, et al.. New distal tibia anterolateral locking plate for internal fixation of Pilon fractures [J]. Chinese Journal of Primary Medicine and Pharmacy, 2010; (4): 455-456.*
11. *Zhan Kaixi, Wang Yijin, Hua Quanke, et al.. Biomechanical study of distal tibia anterolateral anatomical locking plate in treatment of unstable Pilon fractures [J]. Chinese Journal of Bone and Joint Injury, 2010; 25(6): 515-518.*
12. *Musahl V, Tarkin I, Kobbe P, et al.. New trends and techniques in open reduction and internal fixation of fractures of the tibial plateau[J]. Journal of Bone & Joint Surgery, British Volume, 2009; 91(4): 426-433.*
13. *Huang Lei, Zhang Feng, Ye Penghan, et al.. Staging and delayed open restoration and internal fixation in treatment of severe Pilon fractures [J]. Chinese Journal of Orthopaedic Trauma, 2009; (011): 1039-1041.*
14. *Liu Zhiqian, Guo Weihuang, Guo Yingbin, et al.. Discussion of internal fixation approaches of Pilon fractures [J]. Chinese Journal of Bone and Joint Injury, 2012; 27(008): 757-758.*
15. *Wang Xingyuan, Wang Yijian, Fan Yong, et al.. Application of locking plate internal fixation in treatment of Pilon fractures [J]. Chinese Journal of Bone and Joint Injury, 2008; 23(10): 854-855.*


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# Hepatoprotective effects of mushroom (shiitake "lentinus-edodes") against carbon-tetrachloride-induced liver injury in rats

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**Abstract**

**Background and objectives**: Liver injury induced by viruses, toxic chemicals, certain drugs and environmental pollutants, has been on the increase for the past few decades and recognized as a toxicological problem. Carbon tetrachloride is a xenobiotic that produces hepatotoxicity. Mushroom characteristically contains much different biological activities as various degrees of immunity, lowering antitumor and other beneficial or therapeutic health effect without toxicity. The present study was carried out to investigate the hepatoprotective effect of mushroom against carbon tetrachloride intoxication in rats. Dried mushroom (shiitake "*Lentinus-edodes*) grind mushroom at concentration (5 and 10 %) showed significant hepatoprotective activity against carbon tetrachloride induced hepatotoxicity in rats by normalizing the levels of serum AST, ALT, ALP, T. Protein , Albumin , Globulin and A:G ratio. Dried mushroom improved the Glucose, Cholesterol and Triglycerides content levels in a dose dependent manner. Gross necropsy and histopathological examination further confirmed the hepatoprotective effects of mushroom (shiitake "*Lentinus-edodes*). This is the first report on hepatoprotective effects of mushroom (shiitake "*Lentinus-edodes*). The present study showed that mushroom (shiitake "*Lentinus-edodes*) was able to prevent or reduce the severity of carbon tetrachloride -induced liver injury.

**Key words:** Carbon tetrachloride, mushroom, hepatotoprotective, histopathological, liver function.

## Introduction

The liver plays an important role in the detoxification of foreign substances, in the secretion of bile for digestion, and in the metabolic functions of various nutrients including carbohydrates, proteins, and fats Saleem *et al.,* (2010). Hence, chronic liver injury has serious medical consequences. A common chronic disease known as liver fibrosis may lead to end-stage liver cirrhosis and liver cancer Ao *et al.,* (2009). Excessive consumptions of alcohol and viral infections are the most common risk factors for liver diseases in developed countries, while environmental pollution, hepatic viruses, parasitic infections, and chemotherapeutics are the main factors known to cause hepatic damage in developing countries Alshawsh *et al .,* (2011). In spite of medical advances, conventional medicinal approaches have undesirable adverse effects, lack efficiency, and are costly, especially for patients in developing countries Stickel and Schuppan (2007). Elimination of risk factors and alleviation of liver fibrosis are the most common approaches to prevent liver deterioration Brenner *et al.,* (2000). Therefore, there is an urgent need for safe alternative therapeutics to treat liver pathology. Many natural products are being targeted for liver disease prevention and/or treatment Saleem *et al.,* (2010). In recent years, mushrooms have been investigated for their potential for treating liver diseases Ao *et al.,* (2009) and Shi *et al .,* (2008). A mushroom is "a macrofungus with a distinctive fruiting body, which can be either hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand" Chang and Miles, (1992). Mushrooms have been traditionally valued for their nutritional and pharmaceutical properties Omar *et al.,* (2011). Mushrooms have been a major component of Chinese folk medicine since ancient times and it is only quite recently that scientists have begun investigating their bioactive compounds and health modulating mechanisms Lindequist *et al.,* (2005). In

Chinese traditional medicine, mushroom extracts are mixed with herbs in different combinations to treat various medical disorders Diyabalanage *et al.,* (2008). Mushrooms have a huge potential in drug and nutraceutical development. They possess a wide range of pharmacological activities and thus can be considered a functional food. A large number of compounds with antimicrobial, antiviral, antioxidant, antitumor, antiallergic, anti-inflammatory, antiatherogenic, immunomodulating, hypoglycemic, hepatoprotective, and central activities has been characterized and isolated from mushrooms Lindequist *et al.,* (2005) and Preeti *et al.,* (2012). The dried fruiting bodies of mushrooms and extracts of mycelia grown in solid substrate and liquid fermentations are marketed as supplements in the form of powders, capsules, or tablets Wasser, (2005). It was the aim of this work to explore the hepatoprotective effect of Mushroom (shiitake "*Lentinus-edodes*) against Carbon-Tetrachloride-Induced Liver Injury in Rats.

## Materials and Methods

Mushroom white button mushroom (shiitake "*Lentinus-edodes*): Mushroom obtained from Local - market as dry grind before mixed with ration and kept in dry clean plastic bags.

Carbon tetrachloride ($Ccl_4$): Carbon tetrachloride (99.9 purity) was purchased from Sigma Chemical Company. It used in (50ml $Ccl_4$/50 ml propylene glycol) twice/week, subcutaneously injection according to *Borah et al*., (2004).

### *Animals*

Thirty mature male Albino rats of sparague Dawley strain of an average body weight 150 ± 10g were obtained from the laboratory of animal King Fahd Center for Medical Research, Jeddah, Saudi Arabia. Rats were fed on standard ration supplying the essential vitamins, trace elements and water supply was given ad libitum.

### *Experimental design*

Six equal groups each of five rates were housed in wire cages in a room temperature maintained at 25$^{o}$C± 2 and kept under normal healthy conditions. All rats and food consumption weight every week for determination the body weight gain and food intake. Rats of first group (GI) kept as control negative (normal control) and fed on basal ration. Rats of the second group (G2) were used as positive control, fed on basal ration and was injected subcutaneously by (0.1 ml/100 g b. wt.) $Ccl_4$ S / C twice week for two weeks. Rats of the third group (G3) fed on basal ration mixed with grind dried mushroom at concentration 5% for 30 successive days. The fourth Group (G4) fed on basal ration mixed with 10% grind dried mushroom for 30 successive days. The fifth Group (G5) were fed on basal ration mixed with grind dried mushroom at concentration 5% for 30 successive days and at same time injected subcutaneously by (0.1 ml/100 g b.wt.) $Ccl_4$ S/C twice week for two weeks. Rats of sixth group (G6) were fed on basal ration mixed with grind dried mushroom at concentration 10% for 30 successive days and at same time injected subcutaneously by (0.1 ml/100 g b.wt.) $Ccl_4$ S / C twice week for two weeks.

### *Blood samples*

At the end of the experimental period blood samples collected from the animal eye plexuses. Each sample was collected into both heparinized tubes to obtain the plasma and into a free coagulation dry clean centrifuge glass tube to prepare serum. Blood samples were left for15 min at room temperature, and then the tubes were centrifugated for 15 min at 3000 r.p.m for 10 minute. Serum samples were separated and used for determination of different biochemical parameters. After collection of the blood samples, all rats were sacrificed to collect the liver for histopathological.

The gain in the body weights was calculated by the body weight gain = initial weight- final weight.

### *Biochemical parameters*

Serum glucose was determined according to Trinder (1969). Serum cholesterol profile, total (TC) as Waston (1960) and triglycerides (TG) according to Fossati and Prencipe (1982) methods described by, respectively. Liver functions, Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were assayed by the method of Bergmeyer and Harder (1986), alkaline phosphatase (ALP) was measured using the method of Varley *et al*., (1980). Total protein and albumin were determined by using the methods described by Sonnenwirth and Jaret, (1980)

and Drupt (1974). Serum globulin was calculated by subtracting serum globulin from total protein. Albumin/globulin ratio Calculation by division of albumin/globulin.

***Pathological studies***

Liver tissue specimens were collected from all groups at the end of experiments (30 days), 'immediately after sacrifice of rats and fixed in 10% neutral buffered formalin dehydrated in alcohol, cleared in xylol and embedded in paraffin sections of 4µ thickness were prepared, stained by Hematoxylene and Eosin (H&E) (Yoon *et al.*, 2001).

***Statistical analysis***

Results are expressed as mean ± standard deviation (SD). Differences between means in different groups were tested for significance using a one-way analysis of variance (ANOVA) followed by Duncan's test and P value of 0.05 or less was considered significant. Comparative of means were performed according to least significant differences test (LSD) according to (Snedecor, 1969) using SPSS 14 (2006) .

**Results**

The present study was carried out to elucidate the nutritional and protective effect of dried mushroom (shiitake "*Lentinus edodes*) in normal and on carbon tetrachloride ($Ccl_4$) induced cytogenicity and liver injury, using two concentrations of dried mushroom for 30 days in male albino rats. The tested parameters were chemical constituents and histopathological examination of liver in normal rats and rats injected with $Ccl_4$. Their effects and constituents are registered in tables and Photograph.

Effects of injected with $Ccl_4$ on food intake and body weight gain: Effect of feeding on ration mixed with dried mushroom (5 and 10 g / 100 gm ration) for 30 successive days with or without subcutaneous injection of carbon tetra-chloride (twice / week for two weeks) on food intake and body weight gain are recorded in table (1). Food intake and body weight gain was significantly increased in rats feed on ration mixed with dried mushroom (5 and 10 g / 100 g) when compared with other groups, while the group injected $Ccl_4$ showed significantly decreased. The groups feed on dried mushroom at both concentration with injection of $CCl_4$ improved the body weight gain and food intake and showed significantly increased when compared with group subcutaneous injection of $CCl_4$.

Effects of injected with $Ccl_4$ on relative organ weight of liver organ: There was no differences in relative organ weight in rats feed on ration mixed with dried mushroom (5 and 10 %), and rats injected subcutaneously $Ccl_4$ compared with control + ve group (Table1).

Effects of injected with $Ccl_4$ on serum glucose, triglyceride and cholesterol concentration: There was no change in concentrations of serum glucose of rats given dried mushroom, but glucose concentrations were significantly higher in the serum of rats subcutaneously injected with $Ccl_4$. Rats feed on ration mixed with dried mushroom (5 and 10 %) and injected $Ccl_4$ showed significantly decreased in glucose concentration than other groups. Triglyce-

*Table 1. Food intake (g), body weight gain (g) and relative liver weight of rats fed diets containing mushroom without and with $CCl_4$ (n = 5).*

| | Dose (g/100g food) | Food intake (g) | b.wt. gain (g) | Liver % |
|---|---|---|---|---|
| **Control (-ve)** | | 3504.8 ± 70.8[a] | 46.24 ± 1.615[a] | 2.74 ± 0.08 [a] |
| **$Ccl_4$ (+ve)** | 0.1 ml | 2630 ± 75.17[b] | 28.94 ± 1.71[b] | 2.87 ± 0.025 [a] |
| **Mushroom** | 5 | 3876 ± 78.78[ce] | 74.76 ± 1.835[c] | 2.79 ± 0.01 [a] |
| **Mushroom** | 10 | 4454 ± 71.87[d] | 95.54 ± 2.01[d] | 2.28 ± 0.01 [b] |
| **Mushroom + $Ccl_4$** | 5+ 0.1 | 3772 ± 69.531[e] | 53.08 ± 2.505[e] | 2.68 ± 0.007[a] |
| **Mushroom + $Ccl_4$** | 10+ 0.1 | 3960 ± 69.64[e] | 63.7 ± 3.23[f] | 2.702± 0.29 [a] |
| **F-calculated** | | 70.5 # | 109.033 # | 2.822# |
| LSD | | 211.352 | 6.478 | 0.358 |

*# Significant at P < 0.05 using ANOVA test.*

*a, b, c, d, e, f, insignificantly different between two comparison groups within the same letter and column using Least Significant Different (LSD)test at P < 0.05.*

ride and cholesterol concentrations did not induce changes in the serum of rats given dried mushroom than the control –ve group. Serum triglyceride and cholesterol concentrations were increased in groups injected with $Ccl_4$ compared with control –ve group. Groups feed on ration mixed with dried mushroom (5 and 10 %) and injected $Ccl_4$ showed significant decrease in triglyceride and cholesterol values compared with the control group(**(+ve)**. This is showed in Table (2).

Effects of injected with $Ccl_4$ on liver function: The obtained results showed that feeding on ration mixed with dried mushroom (5 and 10 g / 100 g ration) did not induce changes in serum AST , ALT and ALP when compared with control –ve group. The CCl4 intoxication significantly elevated the ALT, AST and highly significant increase in the activity of serum ALP as compared to control group (C –ve) (Table 3). Feeding on ration mixed with dried mushroom (5 and 10 g / 100 g ration) with injected $Ccl_4$ of rats at a dose of 0.1 ml /100 g b.wt. produced significant decrease of AST, ALT and ALP when compared with control group and directed toward normal (C –ve). It was clear from table (3).

Effects of injected with $Ccl_4$ on serum protein: The obtained results showed that feeding on ration mixed with dried mushroom (5 and 10%) did not induce changes of serum total protein, albumin, globulin and A: G ratio when compared with control –ve group. The CCl4 intoxication showed significantly decreased serum protein, albumin, and globulin and A: G ratio. Feeding on ration mixed with dried mushroom ( 5 and 10 % ) with injected $Ccl_4$ caused significant increase in total protein, albumin, globulin and A:G ratio, directed toward normal as compared with control (-v)group (Table 4).

Pathological effects: Figure (1) showed the Control (-ve), untreated rat group revealed a normal histological structure of hepatic lobule (Slide 1). Meanwhile, (Slide 2), rats injected $Ccl_4$ (con-

*Table 2. Mean values of serum glucose, triglycerides and cholesterol in rats fed diets containing mushroom without and with $Ccl_4$ (n = 5).*

| | Dose (g/100g food) | Glucose (mg/dl) | Cholesterol (mg/dl) | Triglycerides (mmol/L) |
|---|---|---|---|---|
| **Control (-ve)** | | 113.52 ±5.66$^{a}$ | 136.00 ±5.05$^{a}$ | 4.38±0.38$^{a}$ |
| **$Ccl_4$ (+ve)** | 0.1 ml | 148.16 ±2.95$^{b}$ | 241.15 ±8.369$^{b}$ | 6.41 ±0.38$^{b}$ |
| **Mushroom** | 5 | 111.2 ±4.37$^{a}$ | 132.88 ±1.227$^{a}$ | 4.29 ±0.22$^{a}$ |
| **Mushroom** | 10 | 113.42 ±6.39$^{a}$ | 146.53 ±10.44$^{a}$ | 4.31 ±0.31$^{a}$ |
| **Mushroom +$Ccl_4$** | 5+ 0.1 ml | 130.04 ±6.7$^{c}$ | 192.93 ±11.06$^{c}$ | 5.36 ±0.27$^{cd}$ |
| **Mushroom + $Ccl_4$** | 10+ 0.1 ml | 104.48 ±5.2$^{a}$ | 148.88 ±15.09$^{a}$ | 4.64 ±0.207$^{ad}$ |
| **F-calculated** | | 8.949# | 19.396# | 7.74# |
| **LSD** | | 15.632 | 28.129 | 0.883 |

*# Significant at P < 0.05 using ANOVA test.*
*a, b, c, d, e, insignificantly different between two comparison groups within the same litter and column using Least Significant Different at P < 0.05.*

*Table 3. Mean activities of transaminases (AST&ALT) and alkaline phosphatase (ALP) in the serum of rats fed diets containing mushroom without and with $Ccl_4$ (n = 5).*

| | Dose (g/100g food) | AST (u/l) | ALT (u/l) | Alkaline Phosphatase (u/l) |
|---|---|---|---|---|
| **Control (-ve)** | | 25.26± 0.327$^{de}$ | 11.896 ± 0.414$^{d}$ | 41.78 ± 1.816$^{d}$ |
| **$Ccl_4$ (+ve)** | 0.1 ml | 49.43 ± 0.232$^{a}$ | 25.645 ± 0.186$^{a}$ | 84.748 ± 2.53$^{a}$ |
| **Mushroom** | 5 | 24.4 ± 0.203$^{d}$ | 11.441 ± 0.089$^{d}$ | 40.76± 2.68$^{d}$ |
| **Mushroom** | 10 | 26.19 ± 0.37$^{e}$ | 10.64 ± 0.119$^{d}$ | 40.55 ± 1.197$^{d}$ |
| **Mushroom + $Ccl_4$** | 5+ 0.1 ml | 33.008 ± 0.36$^{b}$ | 16.42 ± 0.446$^{b}$ | 72.839 ± 2.5$^{b}$ |
| **Mushroom + $Ccl_4$** | 10+0.1ml | 31.27 ± 0.579$^{c}$ | 14.22 ± 0.962$^{c}$ | 51.25 ± 1.64$^{c}$ |
| **F-calculated** | | 657.463# | 142.115# | 79.37# |
| **LSD** | | 1.067 | 1.383 | 6.2096 |

*# Significant at P < 0.05 using ANOVA test.*
*a, b, c, d, e, insignificantly different between two comparison groups within the same letter and column using Least Significant Different at P < 0.05.*

*Table (4). Mean activities of liver function, total serum protein, albumin, globulin and albumin: globulin ratio (A: G ratio) in the serum of rats fed diets containing mushroom without and with $Ccl_4$ (n = 5).*

| | Dose (g/100g food) | T. Protein (g/dl) | Albumin (g/dl) | Globulin (g/dl) | A:G ratio |
|---|---|---|---|---|---|
| **Control (-ve)** | | 7.47 ± 0.396 [a] | 3.76± 0.33 [ac] | 3.64 ± 0.098 [ac] | 1.126± 0.096[ac] |
| **$Ccl_4$ (+ ve)** | 0.1 ml | 5.21 ± 0.43 [b] | 2.21 ± 0.15 [b] | 2.757 ± 0.199[b] | 0.764 ± 0.064[b] |
| **Mushroom** | 5 | 7.12 ± 0.59 [a] | 3.995 ± 0.16 [a] | 3.32 ± 0.17[a] | 1.363 ± 0.13[ac] |
| **Mushroom** | 10 | 7.41 ± 0.5 [a] | 3.83 ± 0.28 [ad] | 3.438 ± 0.13[a] | 1.09 ±0.087[abc] |
| **Mushroom + $Ccl_4$** | 5+ 0.1 ml | 6.77 ± 0.33 [a] | 3.195 ± 0.22[cd] | 3.593 ± 0.12[a] | 0.89 ± 0.138[ac] |
| **Mushroom + $Ccl_4$** | 10+0.1 ml | 7.16 ± 0.66 [a] | 3.43 ± 0.2 [a] | 3.773 ± 0.13[c] | .954 ± 0.058[ab] |
| **F-calculated** | | 2.885# | 7.889# | 6.147# | 2.986# |
| **LSD** | | 1.447 | 0.678 | 0.424 | 0.339 |

*# Significant at P < 0.05 using ANOVA test.*
*a, b, c, d, e, insignificantly different between two comparison groups within the same litter and column using Least Significant Different at P < 0.05.*

trol + ve) showed liver necrosis and fibrosis of the centrilobular hepatocytes with of focal areas of intracytoplasmic vacuolar degeneration, whereas liver of rat from all groups mushroom + $Ccl_4$ showed, slight kupffer cells activation (Slide 3). In addition, liver of rat from all groups mushroom showed no changes with apparent normal hepatocytes (Slide 1).

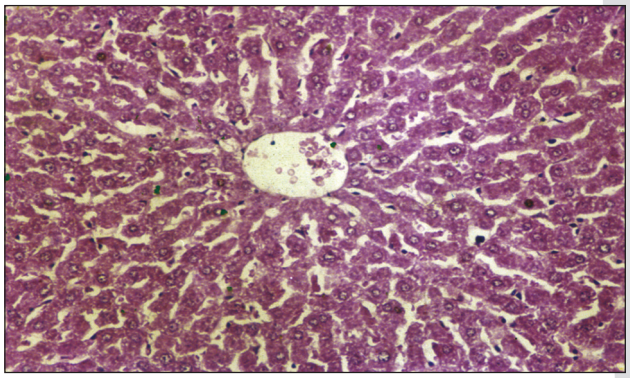

*Figure 1. Control (-ve)*

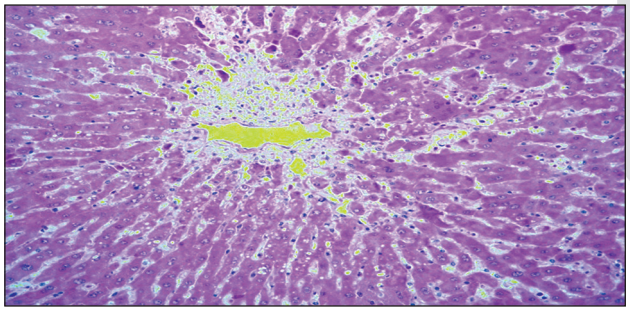

*Figure 2. Control (+ve)*

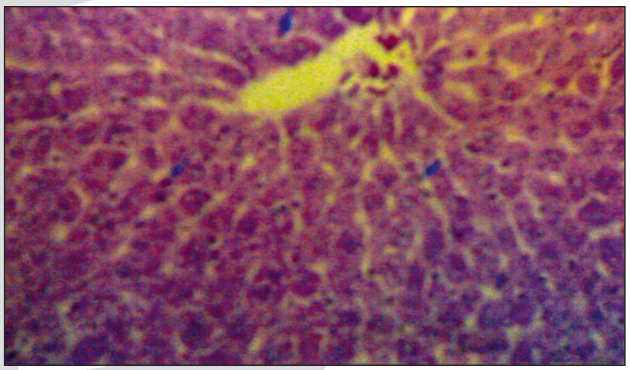

*Figure 3. Mushroom (5 or 10 gm) + $Ccl_4$*

## Discussion

Mushroom (*Lentinus edodes*) has long been known to be endowed with beneficial and diverse activity. The present study was carried out to elucidate the hepatoprotective effect of Mushroom (shiitake "*Lentinus-edodes*) against Carbon Tetrachloride-Induced Liver Injury in Rats.

It is generally believed that carbon tetrachloride ( $Ccl_4$ ) hepatotoxicity results from the bioactivation of the $Ccl_4$ molecules to the trichloromethyl toxic free radical by certain isozymes of cytochrome P450 ( CYP – 450 ). Once the trichoromethyl radical is formed, it reacts with molecular oxygen to form the highly toxic peroxy radical which then attacks cell membrane lipids to propagate a chain reaction leading to initiation of lipid peroxidation and break down of membrane structure (Youssef, 2000 and Kalava and Menon 2012).

Effects of injected with $Ccl_4$ on food intake and body weight gain: Feeding of dried mushroom at both concentrations for 30 successive day's signifi-

cant increase in body weight gain and food intake. These findings correlated with those obtained by Hush *et al.,* (2006), but our results were disagree with the results of Fukushima *et al.,* (2001) who reported that there did not change in body weight gain and Hong *et al.,* (2007) who recorded that feed on mushroom decrease body weight in mice and rats. Subcutaneous injection of $Ccl_4$ caused a significant decrease in body weight gain and food intake. These results are in agreement with Lee *et al.,* (2007) who reported that injection of $Ccl_4$ was significantly decreased body weight gain and food intake. Feeding on dried mushroom with injection $Ccl_4$ seemed to alleviate the inhibitory effect of $Ccl_4$ on body weight gain and foods intake. Moreover, the beneficial effect of antioxidant administration against $Ccl_4$ poisoning with respect to body weight observed in the present study confirms previous results obtained by Aneja *et al.,* (2005) who concluded that feeding rats with antioxidants could play an important role as a prophylactic against the toxic effects of $Ccl_4$. Hesx *et al.,* (2006) found that mushroom contain several compound such as polysaccharide, polysaccharide – peptide complex and phenolic components proposed and flavonoids to be responsible for this antioxidant effect.

Effects of injected with $Ccl_4$ on serum glucose, triglyceride and cholesterol concentrations: The present results revealed that was no changes in concentrations of serum glucose of rats given dried mushroom, while glucose concentrations were significantly higher in the serum of rats subcutaneously injected with $Ccl_4$. On other hand rats feed or ration mixed with dried mushroom (5% and 10%) with injected $Ccl_4$ showed statistically decreased glucose concentration than control + ve group($Ccl_4$ alone). Our results are disagree with Yang *et al.,* (2006) and Khatun *et al.,* (2007) who reported that mushroom significantly reduced blood glucose, without any deleterious effect on liver and kidney. The effect of mushroom may be investigated in a large sample for a longer duration to evaluate its efficacy and toxicity, while Hemmings and Song (2005) who found that $Ccl_4$ (alone) treatment effected a 25% reduction in plasma glucose levels.

Triglyceride and cholesterol concentrations did not induce changes in the serum of rats given dried mushroom than the control –ve group. Our results are in agreement with Hong *et al.,* (2007) and Khatun *et al.,* (2007), concerning the changes in triglyceride and cholesterol concentration a significant decrease were recorded in hypercholesterolemia of mushroom. The present results revealed that triglyceride and cholesterol concentration showed significantly increased in serum of rats' injected $Ccl_4$, this result agreement with Abdel-Hamid (2006) study which observed increase in the concentrations of triglyceride and cholesterol in the serum of rats injected with $Ccl_4$.

Effects of injected with $Ccl_4$ on liver function: On regard to the present study, feeding on ration mixed with dried mushroom at both concentrations ( 5 and 10 g / 100 g ration ) did not induce changes in AST and ALT when compared with control –ve group, these results are in agreement with JayaKumar *et al.,* (2006) and Lakshmi *et al.,* (2006) who found administration of mushroom to male wister rats for 4 days resulted in significantly elevated in GOT and GPT and in agreement with Yang *et al.,* (2007) who reported that administration of GAE & CCE showed decrease in the activities of AST and ALT. Injection of rats with $Ccl_4$ led to significant increase of both AST and ALT enzymes, as compared to control group (C –ve). It is believed that the most accepted hypothesis of hepatotoxicity for $Ccl_4$ is the bioactivation of $Ccl_4$ molecules to the trichloromethyl toxic free radical by certain is coenzymes of cytochrome P – 450. When $Ccl_3$ is formed, it leads to lipid peroxidation of the polyunsaturated fatty acid in cell membranes, break down of membrane – structure and leading to the release of microsomal corboxyal esterase and other enzymes, such as amino transferases in to the extra cellular compartments including serum (Wong *et al.,* 1998 ). These results agree with previous studies of Jayakumar *et al.,* (2006) and Raja *et al.,* (2007). Who reported that feeding on *Ginkgo biloba*, shiitakii, *Cytisus scoparius* and Oyster mushroom with injection of $Ccl_4$, there was a remarkable improvement of AST and ALT levels and revealed to near normal. These results suggest that feeding on mushroom is able to significantly alleviate the hypatotoxicity induced by $Ccl_4$ in the rat.

Serum ALP activity: Alkaline phosphatase activity in groups feeding on ration mixed with dried mushroom NOAEL (no observable effective level) changes for serum ALP compared to control –ve group, these results are agreement with Hesx

*et al.,* (2006) and Jayakumar *et al.,* (2006) who reported that feeding on mushroom (*Ginkgo biloba*), oyster mushroom and *Ganoderma lucidum* extract tend to insignificant changes in hepatic enzyme. Increased with rats injected $Ccl_4$ and this is agreement with Ogeturk *et al.,* (2004).

In general alkaline phosphatase (ALP) is present in nearly all tissues of the body utilizing glucose for energy, so it is in the group with low specificity. It is present at high concentration in osteoblasts, liver, gastrointestinal mucosa, renal tubules and spleen, so its elevation may be due to liver diseases, renal diseases or gastrointestinal lesions (Benjamin, 1979).

Feeding of dried mushroom with injected $Ccl_4$ significantly decreased the ALP activity when compared with control + ve group ($Ccl_4$ group). These results may attribute to the presence of antioxidants of mushroom which had important beneficial effects on the liver regeneration Sun *et al.,* (2004) and Hesx *et al.,* (2006).

Effects of injected with $Ccl_4$ on serum protein: Feeding on ration mixed with dried mushroom (5% and 10%) did not induce changes of serum total protein, albumin, globulin and A: G ratio when compared with control –ve group. These results are agreement with Hesx *et al.,* (2006) studied the effect of extract from *Ginkgo biloba* in rats for 7 weeks. The results indicate that the level of albumin was notably and Lin and Lin (2006) who found that administration of Reishi mushroom and *Ganoderma lucidum* extract in rats for 8 weeks showed no changes in level of albumin and albumin / globulin ratio.

Injection of $Ccl_4$ Subcutaneously caused significantly decreased in serum total protein, albumin and A: G ratio. Our results agree with Li *et al.,* (2004) who found that subcutaneous injection of $CCl_4$ for 3 months to wister rats showed significantly decreased the level of albumin and A / G ratio. And results are in agreement with those reported by Li et *al.,* (2004) and Ogeturk *et al.,* (2004).

From the above mentioned results, it is easy to notice that $CCl_4$ clearly affect the liver and its enzymes and this negative effect decrease the liver capacity to synthesis the proteins and also mushroom contain antioxidants and flavonoids which improved nutritional status as anti-inflammatory and beneficial effect on the liver protection **[**Lin *et al.,* 2006 and Watanabe *et al.,*2006].

In conclusion, the results in this study showed significant decreases in total protein, albumin and A: G ratio, which may be due to impaired kidney and liver function. Because albumin is synthesized by the liver, decreased albumin may result from liver damage. It can also result from kidney disease, which allows albumin to escape into the urine **(**Kaneko *et al.,* 1997) and also, the major site of synthesis of the plasma proteins is the liver and the second major site is the immune system. Rats feed on ration mixed with dried mushroom and injected $Ccl_4$ showed significant increase in total protein, albumin and A: G ratio directed toward normal value. The obtained data are agreement with Hesx *et al.,* (2006); Lin and Lin (2006) and Lin *et al.,* (2006) who reported that feed on Reishi mushroom, mycelia from *Antrodia camphorata* and *Ginkgo biloba* to rats showed increase in the level of protein, albumin and A : G ratio directed toward normal. This is useful for reduces chronic liver injury and liver fibrosis in rats with liver injury induced by $Ccl_4$.

Pathological effects of liver organs:

There were different pathological effects on internal organs as follows: rats injected with $CCl_4$ ( control + ve ) showed liver necrosis and fibrosis of the centrilobular hepatocytes with focal areas of intracytoplasmic vacular degeneration and diffuse vacuolar degeneration and diffuse vacuola, degeneration with enzyme induction of the most of the hepatic cells.

Our results are in agreement with Fang *et al.,* (2007) and Mendoza *et al.,* (2007), who reported that the injuries (experimental group) were induced by subcutaneous injections of $Ccl_4$ in a 50% olive-oil solution occurred swelling of the liver and multiple nodulai formation of pseudolobules indicated a form of liver cirrhosis. Feeding of mushroom with injection of $Ccl_4$ are in agreement with Li *et al.,* (2004) who reported that feed on mushroom ( bicyclol alleviates) has protective effect on liver fibrosis. The liver transforming growth factor beta 1 (TGF beta 1) level in the 2 bicyclol group was significantly lower than that of the model group. The fluidity and swelling ability of mitochondrial membrane significantly decreased in group feed on mushroom after injection of $Ccl_4$ for one month to mice and the binding activity of nuclear NF Kappa B remained very high in the model group mice. Bicyclol alleviates $Ccl_4$ induced liver fibrosis by

its anti-peroxidation and anti-inflammation functions and regulation of nuclear factor kappa (NF-kB) DNA – binding activity. Ding *et al.,* (2005) who recorded that the effect of *Ginkgo biloba* (mushroom) on liver fibrogenesis was detected by H&E staining. The results showed significantly inhibited TGF – beta 1 and collagen 1 expression in rat liver. Lin and Lin (2006) found that the oral administration of Reishi mushroom and *Granoderma lucidum* extract significantly reduced the liver fibrosis induced by $Ccl_4$, in rats, probably by exerting a protective effect against hepatocellular necrosis by its free radical scavenging ability. Lin *et al.,* (2006) who reported that administration of *Antrodia camphorata* on liver fibrosis induced by $Ccl_4$ in rats showed less fibrotic septa than $CCl_4$ group. It recorded that Fermented Mycelia from Antrodia Camphorata (FMAC) can retard the progression of liver fibrosis induced by $CCl_4$ in rats.

## References


1. *Abdel – Hamid NM. Diphenyl Dimethyl bicarboxylate as an Effective treatment for chemical-induced fatty liver in rats. Afr. J. Biomed. Res., 2006; 9: 77 – 81.*

2. *Alshawsh MA, Abdulla MA, Ismail S, Amin ZA. "Hepatoprotective effects of Orthosiphon stamineus extract on thioacetamide-induced liver cirrhosis in rats," Evidence-Based Complementary and Alternative Medicine, 2011; vol. 2011: 6.*

3. *Aneja R, Upadhyaya G, Prakash S, Dass SK, Chandra R. Ameliorating effect of phytoestrogens on $CCl_4$ – induced oxidative stress in the livers of male wistar rats. Artif. Cells Blood Substit. Immobil. Biotechnol., 2005; 33(2): 201 – 13.*

4. *Ao ZH, Xu ZH, Lu ZM, Xu HY, Zhang XM, Dou F. "Niuchangchih (Antrodia camphorata) and its potential in treating liver diseases," Journal of Ethnopharmacology, 2009; 121(2), 194–212.*

5. *Bergmeyer HU, Harder M. A colorimeteric method of determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Clin. Biochem., 1986; 24: 28 - 34.*

6. *Benjamin MM. Outline of Veterinary Clinical. 2nd ed., the Iowa state University Press. Ames, Towa, U.S.A. 1979.*

7. *Borah RC, Mohan P, Choudhury BH, Barua IC. Hepatoprotective activity of phyllanthus fraternusl and glycosmis phentaphylla (Retz) correa used against jaundice in N-E. India. Bioprospecting of commercially important plants proceedings of the national symposium on biochemical approaches for utilization of exploitation of commercially important plants. Jorhat: 2004; 259-262.*

8. *Brenner DA, Waterboer T, Choi SK, et al. "New aspects of hepatic fibrosis," Journal of Hepatology, 2000; 32(1), 32–38.*

9. *Chang ST, Miles PG. "Mushroom biology- A new discipline, "Mycologist, 1992; 6(2), 64–65.*

10. *Ding J, Yu J, Wang C, Hu W, Li D, et al. Ginkgo biloba extract alleviates liver fibrosis induced by $CCl_4$ in rats. Liver International, 2005; 25(6): 1224-1232.*

11. *Diyabalanage T, Mulabagal V, Mills G, DeWitt DL, Nair MG. "Health-beneficial qualities of the edible mushroom, Agrocybe aegerita," Food Chemistry, 2008; 108(1), 97–102.*

12. *Drupt F. Colorimetric method for determination of albumin. Pharm. Biol., 1974; 9: 777-779.*

13. *Fang CH, Liu SJ, Chen XW, Cui B, Wang Y, Huang ZR. The tracking of allogenic grafted rat bone marrow stem cells in rat liver and their role on repairing injured liver Zhonghua Wai Ke Zhi., 2007; 45(9): 598 – 601.*

14. *Fossati P, Prencipe L. The determination of triglycerdes using enzymatic methods. Clin. Chem., 1982; 28: 2077-2081.*

15. *Fukushima M, Ohashi T, Fujiwara Y, Sonoyama K, Nakano M. Cholesterol lowering effects of maitake (Grifola Frondosa) fiber shiitake(Lentinus Edodes) fiber and enokitake (Flammulina Velutipes) fiber in rats. Exp. Biol. Med., 2001; 226(8): 758-765.*

16. *Hemmings SJ, Song X. The effects of dietary flaxseed on the fischer 344 rat. 111. protection against $CCl_4$ – induced liver injury. Cell Biochem. Funct. 2005: 23(6): 389 – 398.*

17. *Hesx LJ, Wang YP, Wang YL, Fu H, Xu JL, Zhao G, Liu EQ. Effects of extract from Ginkgo biloba on carbon tetrachloride - induced liver injury in rats. World J. Gastroenterol., 2006; 12(24): 3924 – 3928.*

18. *Hong L, Xun M, Wutong W. Anti-diabetic effect of an alpha-glucan from fruit body of maitake (Grifola frondosa) on KK-Ay mice. J. Pharm. Pharmacol., 2007; 59(4): 572-582.*

19. *Hush WJ, Lien JL, Jiaw ET, Lee MY. Antihyperlipidemic and antioxidant effects of extracts from pleurotus citrinopileatus. Appl. Microbiol. Biotechnol. 2006; 72(3): 117-123.*

20. *Jayakumar T, Ramesh E, Geraldine P. Antioxidant activity of the oyster mushroom, Pleurotus ostreatus, on $CCl_4$-induced liver injury in rats. Food Chem. Toxicol., 2006; 44(12): 1989-1996.*

21. *Kalava SV, Menon SG. Protective efficacy of the extract of volvariella volvacea (bulliard ex fries) singer. against carbon tetrachloride induced hepatic injury. International Journal of Pharmaceutical Sciences and Research, 2012; 3(8): 2849-2856*

22. *Kaneko JJ, Harvey WJ, Bruss LM. Clinical biochemistry of domestic animals. 5th ed. Academic press, San Diego, London. 1997.*

23. *Khatun K, Mahtab H, Khanam PA, Sayeed MA, Khan KA. Oyster mushroom reduced blood glucose and cholesterol in diabetic subjects. Mymensingh. Med. J., 2007; 16(1): 94- 99.*

24. *Lakshmi B, Ajith TA, Jose N, Janardhanan KK. Antimutagenic activity of methanolic extract of Ganoderma lucidum and its effect on hepatic damage caused by benzo[a] pyrene. J. Ethnopharmacol ., 2006; 107(2): 297-303.*

25. *Lee HS, Keum KY, Ku SK. Effects of picrorrhiza rhizome water extracts on the sub acute liver damages induced by carbon tetrachloride. J. Med. Food., 2007; 10(1): 110 –117.*

26. *Li Y, Li Y, Liu GT. Protective effects of bicyclol on liver fibrosis induced by carbon tetrachloride] Zhonghua. Yi. Xue. Za. Zhi ., 2004; 84(24): 2096-2101.*

27. *Lin WC, Kuo SC, Lin WL, Fang HL, Wang BC. Filtrate of fermented mycelia from Antrodia camphorata reduces liver fibrosis induced by carbon tetrachloride in rats. World J. Gastroenterol., 2006; 12(15): 2369-2374.*

28. *Lin WC, Lin WL. Ameliorative effect of Ganoderma lucidum on carbon tetrachloride-induced liver fibrosis in rats. World J. Gastroenterol., 2006; 12(2): 265-270.*

29. *Lindequist U, Niedermeyer THJ, J¨ulich WD. "The pharmacological potential of mushrooms," Evidence-Based Complementary and Alternative Medicine, 2005; 2(3), 285–299 .*

30. *Mendoza S, Noa M, Pèrez Y, Mas R. Preventive effect of D – 002, a mixture of long – chain alcohols from bess wax, on the liver damage induced with $Ccl_4$ in rats. J. Med. Food., 2007: 10(2): 379 – 383.*

31. *Ogeturk M, Kus I, Kavakli A, Zararsiz I, Ilhan N, Sarsilmaz M. Effects of melatonin on carbon tetrachloride – induced changes in rat serum. J Physiol Biochem., 2004; 60(3): 205 – 210.*

32. *Omar N, Mhd A, Noorlidah Abdullah, Kuppusamy UR, Abdulla MA. "Nutritional composition, antioxidant capacity and antiulcer potential of Lentinus squarrosulus (Mont.) mycelia extract," Evidence Based Complementary and Alternative Medicine, 2011; vol. 2011 , 8.*

33. *Preeti A, Pushpa S, Sakshi S, Jyoti A. Antioxidant mushrooms: review, International Ressearch Journal of Pharmacy, 2012; 3(6): 65-70.*

34. *Raja S, Ahamed KF, Kumar V, Mukheriee K, Bandyopadhyay A, Mukherjee PK. Antioxidant effect of Cytisus scoparius against carbon tetrachloride treated liver injury in rats. J. Ethnopharmacol., 2007; 109(1): 41 – 47.*

35. *Saleem M, Madhusudhana Chetty S, Ramkanth S, Rajan VST, Mahesh Kumar K, Gauthaman K. "Hepatoprotective herbs—a review," International Journal of Research in Pharmaceutical Sciences, 2010; 1(1), 1–5.*

36. *Shi Y, Sun J, He H, Guo H, Zhang S. "Hepatoprotective effects of Ganoderma lucidum peptides against d-galactosamine-induced liver injury in mice," Journal of Ethnopharmacology, 2008; 117(3), 415–419 .*

37. *Snedecor GW. Statistical methods "Fourth Ed.; The lowa state, college press, Ames lowa, 1969.*

38. *SPSS 14 "Statistical Package for Social Science, SPSS for windows Release 14.0.0, 12 June, 2006." Standard Version, Copyright SPSS Inc., 1989-2006, All Rights Reserved, Copyright ® SPSS Inc, 2006.*

39. *Sonnenwirth A, Jaret L. Grad wholes clinical laboratory mehods and diagnosis. vol. 18th ed Mosby. London, 1980.*

40. *Stickel F, Schuppan D. "Herbalmedicine in the treatment of liver diseases," Digestive and Liver Disease, 2007; 39(4), 293–304 .*

41. *Sun J, He H, Xie BJ. Novel antioxidant peptides from fermented mushroom Ganoderma lucidum. J. Agric. Food Chem., 2004; 52(21): 6646- 6652.*

42. *Trinder P. Determination of glucose in blood using glucose oxidase with an altemative oxygen acceptor. Am. Clin. Biochem., 1969; 6: 24-27.*

43. *Varley H, Gewenlock A, Bell M. Practical Clinical Biochemistry. 1980; Vol. 1. 5th ed., 741, 897. London: William Heinemen Medical. Books, Ltd.*

44. *Wasser SP. "Reishi or lingzhi (Ganoderma lucidum)," in Encyclopedia of Dietary Supplements, 2005; 603–622, Marcel Dekker, Berlin, Germany.*

45. *Waston DA. Simple method for the determination of serum cholesterol. Clin. Chem. Acta., 1960; 5: 589-596.*

46. *Watanabe A, Kobayashi M, Hayashi S, Kodama D, Isoda K, et al. Protection against D-galactosamine-induced acute liver injury by oral administration of extracts from Lentinus edodes mycelia. Biol. Pharm. Bull., 2006; 29(8): 1651-1654.*

47. *Wong BH, Zuzel KA, Rahman K, Billington D. Protective effect of aged garlic extract against bromobenzene toxicity to precision cut rat slices. Toxicology., 1998; 126(3): 213-222.*

48. *Yang BK, Jung YS, Song CH. Hypoglycemic effects of Ganoderma applanatum and Collybia confluens exo-polymers in streptozotocin-induced diabetic rats. Phytother. Res., 2007; 21(11): 1066-1069.*

49. *Yang K, Jeong SC, Lee HJ, Sohn DH, Song CH. Antidiabetic and hypolipidemic effects of Collybia confluens mycelia produced by submerged culture in streptozotocin-diabetic rats. Arch. Pharm. Res., 2006; 29(1): 73-79.*

50. *Yoon BI, Choi YK, Kim DY, Hyun BH, Joo KH, Rim HJ, Lee JH. Infectivity and pathological changes in murine clonorchiasis: Comparison in immunocompetent and immunodeficient mice. J. Vet. Med. Sci., 2001; 63(4): 421-425.*

51. *Youssef AS. Comparative histopathological and immunohistochemical studies on the hepatoprotective activity of garlic and curcumin. Egyp. J. Comp. Path. And Clinic. Path., 2000; 13(2): 1-11.*

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# The Comparision of Hepatitis C Virus (HCV) Core Antigen and HCV RNA Levels for Diagnosis of HCV Infection in Haemodialysis Patients

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**Abstract**

The diagnosis and monitoring of HCV infection are commonly based on anti-HCV assays. But anti-HCV assays indicate only the exposure of virus therefore confirmation with nucleic acid amplification assays is supposed. Architect HCV Ag assay is recently developed for detecting HCV nucleocapsid antigen (HCV Ag) in serum and plasma. We aimed to evaluate the usefulness of HCV Ag test for the diagnosis of HCV infection and to determine the correlation between HCV Ag test and quantitative HCV RNA assay in haemodialysis (HD) patients. Three hundred fifty-six HD patients were included in the study. Anti-HCV assay, HCV Ag test and HCV RNA were studied in all sera. A significant relationship was determined between HCV RNA positivity and HCV Ag positivity by Chi-spuare test ($p<0.001$). The HCV Ag levels were correlated with HCV RNA levels (Spearman test coefficient 0.867, $p<0.001$). The diagnostic sensitivity and specificity of HCV core Ag assay compared to the HCV RNA assay were 96.15% and 100%, respectively. Architect HCV Ag assay is high sensitive and specific to diagnose of HCV infection. Therefore, HCV Ag assay can be used alternative to HCV RNA assays, especially in immunocompromised individuals as HD patients.

**Key words:** HCV, core antigen, HCV RNA, haemodialysis

## Introduction

HCV is a global health problem that affects at least 170 million people arround the world, approximately 3% of world population [1-4]. The seroprevalence of HCV is 0,6% in general population in Turkey [5]. HCV infection occurs among the people who were infected with HCV at a rate of 15-40%. Over 250 thousand people die from HCV-related chronic liver diseases every year [4,6]. The main transmission route of HCV is blood and transfusion of blood products, as well as sexual, intrauterine and nosocomial transmission in the course of dialysis, colonoscopy and surgical procedures are reported [4,6].

HCV is a single strained RNA virus that belongs to the Flaviviridae family [4]. HCV has an icosahedral capsid contained within the viral envelope. The capsid is performed by polymerization of HCV core protein. The HCV core protein is a 21-kd phosphoprotein made of the first 191 amino acids of the polyprotein. In the cytoplasm of infected cells, HCV core protein is setting very closely to the perinuclear membranes and the endoplasmic reticulum, where it polymerizes in the presence of genomic RNA to form viral capsids. The HCV core protein is antigenic, interacts with numerous cellular proteins, induces spesific cellular and humoral responses and plays an important role in the pathogenesis of HCV infection [7-9].

The diagnosis and monitoring of HCV infection are commonly based on anti-HCV assays in routine procedures [2,10-13]. Today third generation ELISA tests, which can detect the antibodies developed against core, NS3 and NS5 region of HCV, are in use. HCV antibodies can be detected in 2-8 weeks after acute infection and persist for life. For this reason, anti-HCV testing can not distinguish the acute and chronic infection, only indicates the exposure of virus [13,14]. Active HCV infection is defined as the presence of HCV RNA in peripheral blood [16-17]. Qualitative and quantitative molecular tests have been developed for screening HCV RNA in peripheral blood. HCV RNA levels can be quantita-

ted by signal amplification techniques as branched DNA method or target amplification techniques as PCR. Especially in developing countries, molecular assays are expensive, time-consuming and requires qualified personnel therefore the use of these tests in routine practice and in blood donors are not suitable [13,17,18].

Recently, assays for detecting HCV Ag in serum and plasma were developed. Studies are performed in which the performance of HCV Ag assay for the diagnose of active HCV infection and the response to the antiviral therapy were evaluated [10,11,13,17,19].

In this study we aimed to evaluate the sensitivity and the specificity of a new, fully automatized chemiluminescent microparticle Immunoassay (CMIA), to determine the correlation between HCV Ag and HCV RNA concentrations in a group of HD patients who have an increased risk for HCV infection.

## Materials and methods

### *Blood Samples*

This study is performed in Antalya, Turkey. The serum samples were collected from 356 HD patients who have sustained HD in Antalya Education and Research Hospital HD Unit and in four private HD centers in Antalya, in June 2011-May 2012. The sexuality, age, the reason of the renal failure, the history of blood transfusion, the onset date of HD, ALT and AST levels of patients were noted.

The blood samples were collected at the begining of HD. Serum and plasma fractions of blood were seperated and the samples were stored at -80°C. Anti-HCV assay (Cobas E601 Analyser, Roche Diagnostics, Mannheim, Germany) and HCV Ag assay (Architect HCV Ag Reagent Kit, Abbott Diagnostics, Germany) were studied in all sera, HCV RNA was studied from plasma by Real Time PCR (Abbott Real Time HCV, Abbott Diagnostics, Germany) technique at the same time. All assays were performed with original kits according to the manifacturer's recommendation.

### *Anti-HCV screen test*

All sera were analyzed by using the commercially available anti-HCV automatized system. The Elecsys anti-HCV assay is an Electrochemiluminescence Immunoassay (ECLIA) for the qualitative detection of total antibodies to HCV in human serum or plasma (Cobas 6000, Roche Diagnostics, Manheim, Germany). The assay is a third generation test that uses peptides and recombinant antigens representing core, NS3 and NS4 proteins for the determination of anti-HCV antibodies. Results are determined automatically by the Elecsys software by comparing the electrochemiluminescence signal obtained from the sample with the cut-off value obtained by anti-HCV calibration. The result $\geq 1.0$ is considered reactive for anti-HCV.

### *HCV Ag assay*

HCV Ag was performed by using the Abbott Architect HCV Ag assay. The Abbott Architect HCV Ag assay is a two-step CMIA for the quantitative determination of core antigen to HCV in human serum and plasma. The assay uses acridinium labeled murine anti-HCV antibodies in the liquid phase and monoclonal anti-HCV coated paramagnetic microparticles as the solid phase. It is dedicated to the Abbott Architect i2000 module with Architect System Software version 5,0. The sample volume required is approximately 110 µl and the total time required for the assay is 35-40 minutes. The cut-off value is 3.00 fmol/liter ( 0.06 pg/ml). The sample value > 3.00 fmol/liter is considered reactive, sample value < 3.00 fmol/liter is considered nonreactive. If the sample value is equal to 3.00 fmol/liter, the test is repeated.

### *HCV RNA Assay*

The Abbott Real Time HCV Assay uses RT-PCR technology combined with homogeneous real time fluorescent detection for the quantitation of HCV RNA. The selection of a conserved region of the HCV genome provides for the detection of genotypes 1, 2, 3, 4, 5 and 6. The assay results are reported in International Units/mL (IU/mL). The upper limit of quantitation for the Abbott Real Time HCV Assay is 100.000.000 IU/mL (8.00 log IU/mL) and the lower limit of quantitation is 12 IU/mL (1.08 log IU/mL).

### *Statistical analysis*

Statistical analysis was performed with SPSS statistical software, version 16.0 (SPSS Inc., Chicago, IL) and p value of <0.05 was considered to

be significant. The sensitivity, specificity, Chi-square test and Spearman's correlation test were used for statistical analysis of the data.

## Results

The study group is consisted of 356 HD patients. There were 190 men (53,45%) and 166 women (46,6%) in the study group. The mean age was 56,93±15,09. Among 356 specimens tested, 52 samples (14.60%) were reactive and 304 samples (85.40%) were non reactive for HCV RNA. All HCV-RNA-non reactive samples were retested with a new sample four weeks later. Three hundred and four HCV RNA-non reactive samples were also negative for HCV Ag (Table 1). HCV Ag was found to be negative in two of 52 HCV RNA-reactive samples. These samples had low viremia (one sample with HCV RNA at level 87 IU/ml and one sample with 882 IU/ml). Fifty cases reactive both HCV RNA and HCV Ag were also positive with anti-HCV test. Anti-HCV was positive in 98 cases of 356 HD patients (27.52%). Fourty-four (44,90%) of 98 patients (reactive for anti-HCV) were women.

*Table 1. HCV Ag and HCV RNA results in 356 serum samples of HD patients.*

| | HCV RNA | | |
|---|---|---|---|
| HCV Ag | Positive | Negative | Total |
| Reactive | 50 | 0 | 50 |
| Non reactive | 2 | 304 | 306 |
| Total | 52 | 304 | 356 |

A significant relationship was determined between HCV RNA and HCV Ag positivity by Chi-square test ($p<0.001$). The HCV RNA and HCV Ag levels were significantly correlated in 50 samples that were reactive for both tests (Figure 1). The Spearman test coefficient was 0.867 ($p<0.001$). The diagnostic sensitivity and specificity of HCV Ag assay compared to the HCV RNA assay were 96.15% and 100%, respectively.

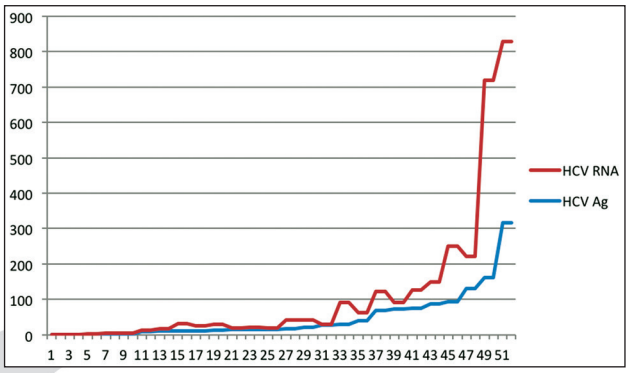


*Figure 1. Relationship between HCV core Ag and HCV RNA results (HCV RNA concentrations were log transformed prior to analysis)*

## Discussion

HD patients have an increased risk for transmission of blood-born infections as HCV depending on the HD procedures and transfusions [17,20,21]. The seroprevalence of HCV in HD patients varies from country to country but it is a fact that it is much higher from general population [22,23]. In Turkey, the seroprevelance of HCV changes from 14% to 83% in HD patients [5].

Anti-HCV assays based on CLIA or ELISA methods are used for the diagnose of HCV infection commonly in Turkey and in the world [19,23]. But there are some disadvantages of anti-HCV assays as; the ratio of false positivity is high (approximately 35%), it can not determine the infection in the window period (45-68 days), there can be false negative results in immunocompromised patients depending on the defect or insufficiency of antibody response [13,19,24,25]. Also anti-HCV assays can not distinguish the individuals who have resolved HCV infection from the patients with active infection [1,12,23,25].

If the anti-HCV result is reactive and concurrently HCV RNA is non reactive in individuals who have low risk for HCV infection, confirmation with RIBA assay is suggested [12,26,27]. But RIBA assays are not useful for confirmation of low positive test results [12]. For this reason, anti-HCV test results and sometimes RIBA test results should be confirmed with HCV RNA assay [11,12, 25].

HCV RNA assays are not suitable for routine utilizations because nucleic acid amplification tests are more expensive and time-consuming, also need qualified personel and exclusive laboratory

*Table 2. The sensitivity and specificity of Architect HCV Ag assay in different studies*

| Study | Country | Year | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|
| Kizilates F | Turkey | 2011 | 96,1 | 100 |
| Park Y | Korea | 2010 | 90,2 | 100 |
| Ross RS | Germany | 2010 | 95 | 100 |
| Ergunay K | Turkey | 2011 | 75,8 | 95,1 |
| Kesli R | Turkey | 2011 | 96,3 | 100 |
| Leary TP | USA | 2011 | 97 | 99 |

settings [2,13,19,29], especially in developing countries. Recently, HCV Ag assays are used as alternative to HCV RNA assays. HCV Ag assay is first developed by Tanaka and et all. in 1995; today fully automatized commercial kits to detect HCV Ag in serum or plasma are available [22,29]. Architect HCV Ag assay is also fully automatized, easy to performe and takes a short time for resulting. In literature there are studies that correspond Architect HCV Ag assay and HCV RNA assays [2,13,17,19,22].

In our study the sensitivity and specificity of Architect HCV Ag assay were found 96.15% and 100% respectively. In some other studies which were performed by the same assay, similar results were determined [1,11,17,19,22]. Table 2 summarized the sensitivity and specificity of Architect HCV Ag in different studies.

In this study, the HCV RNA and HCV core Ag levels were significantly correlated in samples that were reactive for both tests. The Spearman test coefficient was 0.867 ($p<0.001$). The correlation between these tests were found; 0,915 ( $p<0.001$) by Ergunay and et al. , 0,904 ( $p<0.001$) by Miedouge and et al. ,0,907 ( $p<0.001$) by Kesli and et al. , 0,857 ( $p<0.001$) by Ross and et al., 0,740 ( $p<0.001$) by Morota and et al., 0,946 ( $p<0.001$) by Park and et al.

As a result, Architect HCV Ag assay is high sensitive and specific to diagnose of HCV infection, cost-effective, not required special laboratory settings and the procedure takes a short time. Therefore, HCV Ag assay can be used alternative to HCV RNA assays, especially in immunocompromised individuals as HD patients.

## References


1. *Leary TP, Gutierrez RA, Muerhoff AS, Birkenmeyer LG, Desai SM, Dawson GJ. A chemiluminescent, magnetic particle-based immunoassay for the detection of hepatitis C virus core antigen in human serum or plasma. J Med Virol. 2006; 78: 1436-1440.*
2. *Morota K, Fujinami R, Kinukawa H, Machida T, Ohno K, Saegusa H, Takeda K. A new sensitive and automated chemiluminescent microparticle immunoassay for quantitative determination of Hepatitis C virus core antigen. J Virol Methods. 2009; 157: 8-14.*
3. *Simmonds P. Genetic diversity and evolution of hepatitis C virus-15 years on. J Gen Virol. 2004; 85: 3173-3188.*
4. *Richter SS. Laboratory assays for diagnosis and management of hepatitis C virus infection. J Clin Microbiol. 2002; 40: 4407-4412.*
5. *Ozaras R. Hepatit C'de güncel yaklaşimlar. Flora. 2005; 10: 155-162.*
6. *Lauer GM, Walker BD. Hepatitis C virus infection. N Engl Med . 2001; 5: 41-52.*
7. *Bouvier-Alias M, Patel K, Dahari H, Beaucourt S, Larderie P, Blatt L, et al. Clinical utility of total HCV core antigen quantification: a new indirect marker of HCV replication. Hepatology.2002; 36: 211-218.*
8. *Reed KE, Rice CM. Overview of hepatitis C virus genome, structure, polyprotein proceeding and protein properties. Curr Top Microbiol Immunol. 2000; 242: 55-84.*
9. *Lai MM, Ware CF. Hepatitis C virus core protein: possible roles in viral pathogenesis. Curr Top Microbiol Immunol. 2000; 242: 117-134.*
10. *Gretch DR, dela Rosa C, Carithers RL Jr, Willson RA, Williams B, Corey L. Assesment of hepatitis C viremia using molecular amplification technologies: correlations and clinical implications. Ann Intern Med. 1995; 123: 321-9.*

11. *Park Y, Lee JH, Kim BS, Kim do Y, Han KH, Kim HS. New automated hepatitis C virus (HCV) core antigen assay as an alternative to Real-Time PCR for HCV RNA quantification. J Clin Microbiol. 2010; 48: 2253-2256.*

12. *Richter SS. Laboratory assays for diagnosis and management of hepatitis C virus infection. J Clin Microbiol. 2002; 40: 4407-4412.*

13. *Ross RS, Viazov S, Salloum S, Hilgard P, Gerken G, Roggendorf M. Analytical performance characteristics and clinical utility of a novel assay for total hepatitis C virus core antigen quantification. J Clin Microbiol. 2010; 48: 1161-1168.*

14. *Pawlotsky JM. Diagnostic tests for hepatitis C. Hepatol. 1997; 31: 71-79.*

15. *Nakatsuji Y, Matsumoto A, Tanaka E, Ogata H, Kiyosawa K. Detection of chronic hepatitis C virus infection by four diagnostic systems: first-generation and second-generation enzyme-linked immunosorbent assay, second-generation recombinant immunoblot assay and nested polymerase chain reaction analysis. Hepatology. 1992; 16: 300-305.*

16. *Busch MP, Glynn SA, Stramer SL, Strong DM, Caglioti S, Wright DJ, et al. A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors. Transfusion. 2005; 45: 254-264.*

17. *Ergunay K, Sener B, Alp A, Karakaya J, Hasçelik G. Utility of a commercial quantitative hepatitis C virus core antigen assay in a diagnostic laboratory setting. Diag Microbiol Infect Dis. 2011; 70: 486-491.*

18. *Scott JD, Gretch DR. Molecular diagnostics of hepatitis C virus infection: a systematic review (2007). JAMA. 2007; 21; 724-32.*

19. *Kesli R, Polat H, Terzi Y, Kurtoglu MG, Uyar Y. Comparision of a newly developed automated and quantitative hepatitis C virus (HCV) core antigen test with the HCV RNA assay for clinical usefulness in confirming anti-HCV results. J Clin Microbiol. 2011; 49: 4089-4093.*

20. *Ohsawa M, Kato K, Itai K, Tanno K, Fujishima Y, Konda R, et al. Standardized prevalence ratios for chronic hepatitis C virus infection among adult Japanese hemodialysis patients. J Epidemiol. 2010; 20: 303-9.*

21. *Dussol B, Berthezene P, Brunet P, Roubicek C, Berland Y. Hepatitis C virus infection among chronic dialysis patients in the south of France: a collaborative study. Am J Kidney Dis. 1995; 25: 399-404.*

22. *Miedouge M, Saune K, Kamar N, Rieu M, Rostaing L, Izopet J. Analytical evaluation of HCV core antigen and interest for HCV screening in haemodialysis patients. J Clin Virol. 2010; 48: 18-21.*

23. *Fabrizi F, de Vecchi AF, Como G, Lunghi G, Martin P. De novo HCV infection among dialysis patients: a prospective study by HCV core antigen ELISA assay. Aliment Pharmacol Ther.2005; 21: 861-869.*

24. *Glynn SA, Wright DJ, Kleinman SH, Hirschkorn D, Tu Y, Heldebrant C, et al. Dynamics of viremia in early hepatitis C virus infection. Transfusion. 2005; 45: 994-1002.*

25. *National Institutes of Health. NIH consensus statement on management of hepatitis C: NIH Consens. State. Sci. Statements. 2002; 19: 1-46.*

26. *McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, et al. Interferon Alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. N Engl J Med. 1998; 339: 1485-1492.*

27. *Pawlotsky JM, Lonjon I, Hezode C, Raynard B, Darthuy F, Remire J, et al. What strategy be used for diagnosis of hepatitis C virus infection in clinical laboratories? Hepatology. 1998; 27: 1700-1702.*

28. *National Institutes of Health Consensus Development Conference Panel. Management of Hepatitis C. Hepatology. 1997; 26: 2-10.*

29. *Icardi G, Ansaldi F, Bruzzone BM, Durando P, Lee S, de Luigi C, et al. Novel approach to reduce the hepatitis C virus (HCV) window period: clinical evaluation of a new enzyme-linked immunosorbent assay for HCV core antigen. J Clin Microbiol. 2001; 39: 3110-3114.*



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# Therapeutic efficacy of neuromuscular electrical stimulation on post-stroke upper limb spasticity

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## Abstract

**Background:** Spasticity is a frequently observed motor impairment developing after stroke. This study was aimed at evaluating the therapeutic efficacy of neuromuscular electrical stimulation (NMES) on post-stroke upper extremity spasticity and function.

**Methods:** In a randomized clinical trial, 75 patients with post-stroke upper extremity spasticity were studied in Tabriz Imam Hospital. The patients were randomized into three groups; group E received motor level ES on extensor muscles of the affected forearm; group F received sensory level ES on flexor muscles of the affected forearm; and group C or control. ES was applied for 15 days, 0.5 h every day in the case groups. Stretching was also applied for all the studied patients. The grip power, hand index, range of motion (ROM) and Ashworth scale was determined and compared among the three groups at baseline and after treatment.

**Results:** The spasticity improvement was more prominent in group F compared with the controls post-intervention. Improvement of the upper extremity function was better in groups E and F compared with the controls. The gripping force was significantly increased after intervention in group E and to a lesser degree in group F compared with the controls. The active extension were significantly enhanced in group E compared with the group F and controls.

**Conclusion:** NMES as a complementary therapeutic method on the flexor and extensor forearm muscles along with the stretching exercise may improve the post-stroke spasticity and hand function.

**Key words:** Electrical Stimulation, Spasticity, Range of Motion, Grip power.

## Introduction

Spasticity is a frequently observed motor impairment developing after stroke.[1] Following the stroke, approximately 90% of patients experience persistent neurological motor deficits leading to disability and handicap.[2] Nearly 80% of stroke patients survive the acute phase and most patients regain their walking ability. Nevertheless, 30- 66% of the survivors are no longer able to use their affected arm, making them unable to perform the daily activities.[3] The recovery process of function is often slower in the upper extremity compared with the lower extremity.[4]

Critical assessment of the experimental findings indicates that increased excitability of both motor neurons and interneurons plays a crucial role in pathophysiology of the spasticity.[5]

Hand function restoring is difficult following the stroke.[6] Both pharmacological and physical treatment strategies for spastic extremity rehabilitation may be considered.[2] New interventions, such as spinal electrical stimulation (ES) applied to suppress an increased neuronal excitability, may reduce the severity of the spasticity and its complications.[5] ES has been reported to be beneficial for reducing severe hand impairments.[6] It has been demonstrated to provide clinical benefits including improvement of movement, skills and function, as well as decreased spasticity. However, the best methods of treatment accompanying with maximal recovery of the upper limb following the stroke remain uncertain.[7] The effects of ES application during the recovery phase after stroke are also unclear.[7] The purpose of this study was to assess the efficacy of neuromuscular ES in improving the upper extremity spasticity and functional recovery of the acute stroke survivors.

## Methods

This is a randomized clinical trial performed on consecutive patients with spastic paresis of upper extremity following the ischemic stroke, presenting to Physical Medicine & Rehabilitation wards of Tabriz Imam referral hospital. Patients aged between 40 and 60 years, with a history of stroke since 6-24 months prior to the study followed by spastic paresis in the upper limbs were included. Subjects were excluded from the study if they could not tolerate the stimulation, were medically unstable, or had been discharged before the treatment period was completed.

The study was approved by the Regional Ethics Committee. An informed consent was obtained from the patients before the study. The patients were randomly allocated by using computer-generated random numbers and concealed opaque envelops into either E, F, or C groups. Accordingly, group E (n=25) received ES (motor level) in forearm extensor muscles; group F (n=25) received ES (sensory level) in forearm flexor muscles; and group C (n=25) control group. (Fig 1). All three groups concurrently underwent stretching exercises on wrist and finger flexors 3 sessions a day, 4 times in each session, each time lasting for 10 seconds. Electrical stimulation was performed 0.5 hours every day for 15 days in patients of the intervention groups. The intensity of ES in the first group was determined at the level of inducing optimal muscular contraction in each patient.

To assess the involved muscles and severity of spasticity the following parameters were determined before and and following intervention: grip strength (using dynamometer), restoring hand function or hand index using Duruoz hand index (DHI),[8] active and passive wrist range of motion (ROM) (using goniometer), and relief of spasticity or Ashworth scale (through examination). Duruoz hand index is an 18-item questionnaire, with each item scoring 0-5, to evaluate the hand function. Greater score is indicative of more difficulty in the hand function. All of these parameters were evaluated and compared in all groups by a physician who was blind to the type of intervention. Moreover, all of the patients received the same antispastic drug (baclofen tablet). Other studied variables were the type of stroke, side of paresis, age, gender, and duration of the disease.

The ES parameters in group E were pulse current (PC) flow with intensity above motor threshold, pulse duration of 300 μs, frequency of 40 pulses per second, on time of 7 sec, off time of 10 sec, Rx time of 30 min, and electrodes located over the extensor muscles on the posterior aspect of forearm or antagonists to spastic muscles. The ES parameters in group F were as the following: PC flow with intensity below motor threshold, pulse duration of 100 μs, frequency of 60 pulses per second, Rx time of 30 min, and electrodes located over the flexor muscles on the anterior aspect of forearm or spastic muscles.[9]

According to the Ashworth scale,[10] the limb tone was defined as 0 (without increase in tone), 1 (low increase in tone), 2 (marked increase in tone but affected part easily moved), 3 (considerable increase in muscular tone with difficult passive movement of the affected limb), and 4 (affected limb rigid in extension or flexion).

Considering $\alpha$=0.05 and P=4% (response to the therapy), the estimated sample size was 75. Data were analyzed with SPSS-15 statistical software. The collected data were presented as percentage and mean ± standard deviation (SD). Continuous (quantitative) variables were compared by Tukey's test, Paired samples T-test or One-way ANOVA (Independent samples). Categorical (qualitative) variables were compared by contingency tables and Chi-square test or Fisher's Exact Test. *P* value ≤0.05 was considered statistically significant.

## Results

Seventy five patients fulfilling the inclusion criteria were studied in three groups. There was one lost to follow up in group C and two lost to follow up in group F. Demographic characteristics of patients are presented in Table 1. The demographic characteristics were not significantly different among the three studied groups. Table 2 shows the comparison of evaluated parameters in three groups.

In group E, the mean grip power was improved after ES ($P$<0.001). The mean Ashworth scale was decreased after ES ($P$<0.001). The mean hand index was decreased after ES ($P$<0.001). Other changes were not statistically significant.

In group F, the mean active wrist supination was improved after ES ($P$<0.02). The mean grip

*Table 1. Demographic characteristics of patients*

| Variable | Group E | Group F | Group C | P |
|---|---|---|---|---|
| Right side paresis | 13 (52%) | 16 (64%) | 9 (36%) | 0.139 |
| Sex (m) | 12 (48%) | 15 (60%) | 19 (76%) | 0.125 |
| Age (y) | 8.33±55.40 | 9.63±56.68 | 5.50±53.44 | 0.359 |
| Disease duration (m) | 4.11±7.84 | 6.45±10.04 | 2.57±8.88 | 0.254 |

*Table 2. Evaluated parameters in three groups*

| Parameter | Group E | Group F | Group C |
|---|---|---|---|
| Active supination 1 (º) | 36.71±36.80 | 28.29±37.80 | 32.34±46.80 |
| Active supination 2 (º) | 29.35±45.76 | 30.12±45.20 | 31.23±43.80 |
| Active extension 1 (º) | 23.72±12.80 | 19.31±16.00 | 19.04±22.20 |
| Active extension 2 (º) | 26.46±14.00 | 21.98±20.00 | 21.17±22.80 |
| Passive extension 1 (º) | 29.92±35.80 | 24.99±48.20 | 27.37±44.20 |
| Passive extension 2 (º) | 21.47±39.40 | 23.84±51.60 | 27.04±42.20 |
| Grip power 1 | 8.29±17.32 | 12.85±22.80 | 7.71±18.90 |
| Grip power 2 | 7.96±21.06 | 15/12±24.90 | 7.28±17.92 |
| Ashworth scale 1 | 0.93±2.24 | 0.70±2.08 | 0.96±2.40 |
| Ashworth scale 2 | 0.73±1.88 | 0.57±1.92 | 0.84±2.04 |
| Hand Index 1 | 20.25±70.32 | 9.07±73.68 | 6.70±76.64 |
| Hand Index 2 | 23.11±67.44 | 10.53±66.64 | 7.48±78.16 |

*1= before treatment; 2= after treatment*

*Table 3. Changes of studied variables in each group*

| Change | Group E | Group F | Group C | P |
|---|---|---|---|---|
| **Active supination (º)** | 31.2±9.0 | 15.7±7.4 | 9.0±3.0- | 0.094 |
| **Active extension (º)** | 16.9±1.2 | 12.8±4.0 | 8.5±0.6 | 0.625 |
| **Passive extension (º)** | 24.6±3.6 | 35.3±3.4 | 6.5±2.0- | 0.673 |
| **Grip power** | 4.3±3.7 | 1.8±2.1 | 2.3±1.0- | <0.001 |
| **Ashworth scale** | 0.5±0.4- | 0.4±0.2- | 1.0±0.4- | 0.464 |
| **Hand Index** | 4.2±2.9- | 7.4±7.0- | 5.9±1.5 | <0.001 |

*Hand index improved following intervention in groups E and F.*

power was improved ($P$<0.001). The mean Ashworth scale was decreased ($P$<0.04). The mean hand index was decreased after ES ($P$<0.001). Other changes were not statistically significant.

In group C, the changes in the studied parameters were not statistically significant.

Table 3 shows the changes of studied variables in three groups before and after intervention.

Grip power in all evaluations was better in group E than other groups.

## Discussion

In this study the efficacy of neuromuscular ES (ES) on the upper extremity spasticity following stroke was evaluated. In comparison with the control group, ES caused improvement in the upper limb function (using ES at both flexor and extensor levels), grip power (applying ES at flexor and specially extensor levels), spasticity (using ES on the flexor level), and extension (applying ES on the extensor level).

There are numerous studies on the efficacy of ES on spasticity with different etiologies.[11] However; to the best of our knowledge no previous conclusive and randomized controlled clinical trial has been performed to assess the efficacy of ES on the post-stroke upper extremity spasticity. Chae et al. concluded that ES enhanced the upper extremity motor recovery of acute stroke survivors.[11] It has been suggested that ES enhances post-stroke motor recovery, strengthens muscles, and increases the range of motion of joints with prevention or correction of contractures.[7,12] On

the wrist extensors, ES enhances the recovery of isometric wrist extensor strength in hemiparetic stroke patients with some residual motor function at the wrist.[7] Powell et al. found that ES enhanced the recovery of isometric wrist extensor strength at the end of the treatment phase compared with the control group. Enhanced recovery was still apparent at the end of 24 weeks follow-up after ES.[14] Similar findings have been reported in a systematic review of randomized controlled trials of ES applied to various other skeletal muscle groups following stroke.[6,13] These findings are in consistent with the results of our study. In patients with chronic stroke, 20 sessions of a combined ES and TRT (task-related training) home-based program decreased plantar-flexor spasticity, improved dorsi-flexor and plantar-flexor strength, and increased gait velocity significantly more than ES alone, placebo and TRT, or no treatment. Such improvements can even be maintained 4 weeks after treatment is discontinued.[14] Santos et al. used 10 sessions of ES on the forearm flexors and extensors to assist subjects with grasping and releasing a tennis ball. Significant improvement was observed in the Ashworth Scale immediately following intervention. Subjects performed the grasping tasks significantly faster following the interventions. They concluded that severe hand impairment was reduced after a short duration of ES therapy for individuals with chronic stroke.[6] On the other hand, Hara et al. revealed that hybrid ES therapy on the forearm extensor muscles was effective in patients with chronic spastic hemiparesis.[15]

Approximately half of the stroke survivors live with major functional problems in their hands and arms.[7] Following the stroke, motor recovery is enhanced by various techniques.[11] One of the techniques that may facilitate the motor restoration of the stroke survivors is electrical simulation (ES). Temporary reduction of the spasticity has been reported when motor level ES is applied to the antagonists of the spastic muscle. It has been theorized that this is due to post-tetanic depression of reflex pathway or reflex inhibition of the spastic muscle.[9]

Although, our results are in agreement with the aforementioned studies, some of other studies did not find ES as an efficient method for improvement of the post-stroke spasticity.[16,17] These discrepancies may be due to the different study methods in multiple researches, an absence of the control groups or randomization, different therapeutic parameters of ES such as type and their quantitative values, and different tools for an assessment of treatment outcomes.[18] In addition, inefficacy of ES in the spasticity might be due to the fact that spasticity did not occur because of upper motor neuron defects in some studies.[19-21] We found an improvement in the spasticity only when the sensory level ES was performed on the flexor or spastic muscles, in comparison with insignificant results when ES was performed on the extensor or nonspastic muscles.

The mechanism underlying the recovery of neurological injury after stroke has not been completely understood, but more than one process is probably involved.[20] It seems that ES can increase sensory inputs to central nervous system, intensify plasticity, and accelerate the education of motion.[19,21,22] It has been reported that the sensory level ES temporarily reduced the spasticity when delivered to dermatomes of the nerves supplying the spastic muscles and also to skin over the branches innervating the spastic muscles as well as their antagonists.[9] Other mechanism proposed for the reduction of spasticity using the sensory level ES includes a presynaptic inhibition of spastic muscles and activation of descending pathways to the motor neurons of antagonists of the spastic muscles.[9,23,24]

## Conclusion

ES as complementary methods on the flexor and extensor levels in the upper limb along with stretching techniques may improve the post-stroke spasticity. The motor level ES over the extensor level of forearm or antagonist muscles enhances the motor strength and grip power and increases ROM in some directions mainly the active extension, but it has less positive effects on the improvement of hand spasticity. In contrast, the sensory level ES over the flexor level of forearm or agonist muscles decreases the muscle spasticity, but it has lesser effects on the muscle strength. Altogether, it can be assumed that ES application at both extensor and flexor levels can decrease spasticity and improve the upper limb function following stroke. However, due to the presence of multiple functional impairments in stroke, further studies are necessary for prescribing ES for such patients.[25]

## References


1. *Moon SK, Whang YK, Park SU, Ko CN, Kim YS, Bae HS, Cho KH. Antispastic effect of electroacupuncture and moxibustion in stroke patients. Am J Chin Med. 2003; 31(3): 467-74.*
2. *Hesse S, Werner C. Poststroke motor dysfunction and spasticity: novel pharmacological and physical treatment strategies. CNS Drugs. 2003; 17(15): 1093-107.*
3. *Kwakkel G, Kollen BJ, Wagenaar RC. Therapy impact on functional recovery in stroke rehabilitation: a critical review of the literature. Physiotherapy. 1999; 13: 457–470.*
4. *Wagenaar RC. Functional Recovery After Stroke [PhD thesis]. Amsterdam, Netherlands: VU University Press; 1990.*
5. *Elbasiouny SM, Moroz D, Bakr MM, Mushahwar VK. Management of spasticity after spinal cord injury: current techniques and future directions. Neurorehabil Neural Repair. 2010; 24(1): 23-33.*
6. *Santos M, Zahner LH, McKiernan BJ, Mahnken JD, Quaney B. Neuromuscular electrical stimulation improves severe hand dysfunction for individuals with chronic stroke: a pilot study. J Neurol Phys Ther 2006; 30(4): 175-83.*
7. *Powell J. Electrical stimulation of wrist extensors in poststroke hemiplegia. Stroke 1999; 30: 1384-9.*
8. *Duruoz MT, Poiraudeau S, Fermanian J, et al. Development and validation of a rheumathoid hand functional disability scale that assesses functional handicap. J Rheumatol 1996; 23: 1167-72.*
9. *Selkowitz DM. Electrical Currents. In: Cameron MH. Physical agents in rehabilitation. 1st ed. Philadelphia, Saunders.1999; PP: 380-383*
10. *Bohannon RW, Smith MB. Interrater reliability on a modified ashworth scale of muscle spasticity. Physical Therapy. 1987; 67: 206-207.*
11. *Chae J, Bethoux F, Bohinc T, Dobos L, Davis T. Neuromuscular stimulation for upper extremity motor and functional recovery in acute hemiplegia. Stroke 1998; 29: 975-9.*
12. *Chae J, Yu D, Walker M. Percutaneous, intramuscularneuro-muscular electrical stimulation for the treatment of shoulder subluxation and pain in chronic hemiplegia: a case report. Am J Phys Med Rehabil 2001; 80: 296–301.*
13. *Chae J, Bethous F, Bohinc T, Dobos L, Davis T, Friedl A. Neuromuscular stimulation for upper extremity motor and functional recovery in acute hemiplegia. Stroke. 1998; 29: 975–979.*
14. *Ng SS, Hui-Chan CW. Transcutaneous electrical nerve stimulation combined with task-related training improves lower limb functions in subjects with chronic stroke. Stroke 2007; 38(11): 2953-9.*
15. *Hara Y, Ogawa S, Muraoka Y. Hybrid power-assisted functional electrical stimulation improve hemiparetic upper-extremity function. Am J Phys Med Rehabil. 2006 Dec; 85(12): 977-85.*
16. *Skold C, Lonn L, Harms-Ringdahl K, Hultling C, Levi R, Nash M, et al. Effects of functional electrical stimulation training for six months on body composition and spasticity in motor complete tetraplegic spinal cord-injured individuals. J Rehabil Med 2002; 34: 25–32.*
17. *Hines AE, Crago PE, Billian C. Functional electrical stimulation for the reduction of spasticity in the hemiplegic hand. Biomed Sci Instrum 1993; 29: 259–66.*
18. *Low J, Reed A. Electrotherapy explained: principles and practice. Butterworth Heinemann 2006; 5: 220–35.*
19. *Pease WS. Therapeutic electrical stimulation for spasticity: quantitative gait analysis. Am J Phys Med Rehabil 1998; 77: 351–5.*
20. *Weingarden HP, Zeilig G, Heruti R. Hybrid functional electrical stimulation orthosis system for the upper limb: effects on spasticity in chronic stable hemiplegia. Am J Phys Med Rehabil 1998; 77: 276–81.*
21. *Masiero S, Carraro E. Upper limb movements and cerebral plasticity in post-stroke rehabilitation. Aging Clin Exp Res. 2008 Apr; 20(2): 103-8.*
22. *Bogataj U, Gros N, Kljajic M, Acimovic R, Malezic M. Rehabilitation of gait in patients with hemiplegia. A comparison between conventional therapy and multichannel functional stimulation therapy. Phys Ther 1995; 75: 490–502.*
23. *Hazlewood ME, Brown JK, Rowe PJ, Salter PM. The use of therapeutic electrical stimulation in the treatment of hemiplegic cerebral palsy. Dev Med Child Neurol 1994; 36: 661–73.*
24. *Vitenzon AS, Mironov EM, Petrushanskaya KA. Functional electrostimulation of muscles as a method for restoring motor functions. Neurosci Behav Physiol 2005; 35: 709–14.*
25. *Bakhtiary AH, Fatemy E. Does electrical stimulation reduce spasticity after stroke? A randomized controlled study. Clin Rehabil 2008; 22(5): 418-25.*



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# The impact of pandemic on the consumption of influenza antivirals

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**Abstract**

**Objectives:** Influenza is a viral infection that can affect three to five million people in the world each year. Oseltamivir and Zanamivir usage may change during the pandemic

**Methods:** The usage quantities of antivirals were being examined between the years 2007 and 2010, by using the data from Intercontinental Medical Statistics (IMS).

**Results:** Oseltamivir consumption was found as 0,016, 0,022, 0,14, and 0,012 DDD/1000 for the years 2007, 2008, 2009 and 2010, respectively. Zanamivir consumption was found as 0,00000095, 0,000055, 0,0088, and 0.00064 DDD/1000, respectively.

**Conclusion:** There have been significant increase in oseltamivir consumption in year 2009 due to pandemic effect. But same change was not seen for zanamivir consumption. In other years (2007, 2008 and 2010) both antiviral consumption were lower than 2009. The fear and panic caused by a flu pandemic may effect drug consumption substantially.

**Key words:** Influenza, Oseltamivir, Zanamivir

## Introduction

Influenza is an acute viral infection caused by an influenza virus. There are three types of seasonal influenza; A, B and C. Seasonal influenza is mainly caused by Type A and Type B viruses. Type A influenza viruses are further seperated into subtypes according to different kinds and combinations of virus surface proteins. In recent years, infections have been reported due to the influenza A(H1N1) and A(H3N2) subtypes [1].

Influenza epidemics occur yearly during autumn and winter in temperate regions. Three to five million cases of severe illness are reported every year in all over the world. Most of the infected people tend to recover spontaneously within a week or two, but influenza can be fatal among babies and elderly people, and each year it causes 250.000 to 500.000 deaths [1,2].

World Health Organization (WHO) recommends the use of neuraminidase inhibitors (oseltamivir or zanamivir) in the treatment of the uncomplicated influenza for the patients under the risk of severe influenza and complication expansion (babies younger than two years old, adults aged 65 years or older, and pregnant women, those with chronic respiratory, cardiac or metabolic disorders). While WHO recommends oseltamivir treatment for severe or progressive influenza in all patients, they recommend the use of zanamivir for treatment of influenza caused by viruses known to be resistant to oseltamivir. For the patients whose immune systems are suppressed, long term usages of oseltamivir in high doses are being recommended [3].

Oseltamivir was first approved in Switzerland in 1999. Currently it is being used in more than 100 countries and until now more then 83 million patients were treated with it [4]. The use of Zanamivir in the treatment of influenza was approved by FDA in 1999, too [5].

In 2009 an influenza A (H1N1) pandemic was experienced throughout the world. In parallel with the changes in the genetic structure of the virus (schift), the first swine flu case had been reported in Mexico and confirmed in April 2009. After it was shown that the virus had been shedding among people, WHO declared the level of Influenza Pandemic Alarm as a "Phase 5" on 29.04.2009. From the starting date of H1N1 pandemic on 19 April 2009, to February 2010, a total of 529.380 influenza positivism verified with laboratories was declared [6].

Due to this epidemic, the desire in people to learn more about influenza increased and widespread information pollution occurred. For the same epidemic, there was also an increase in the knowledge of the medical staff about the flu and its related side effects. Many clinicians who had administered only symptomatic treatments in flu related cases in previous years preferred to use antiviral treatment during the epidemic. But this epidemic started a panicky environment in the world and the use of vaccine and antivirals were vastly increased.

Even though a wide range of studies pertaining to flu were made during influenza pandemic, in our literature research we could not found any study which were investigating the effects of this epidemic on the consumption of influenza drugs. With this study, we aimed to explore the impact of the 2009 influenza pandemic on the consumption of neuraminidase inhibitors.

## Method

The annual sales figures of antivirals were obtained from Intercontinental Medical Statistics (IMS) Health-Turkey Office [7]. IMS is a market research company which collects drug sales statistics for many countries. The data has been collected between the dates of 2007-2010, by using the defined daily dose (DDD) measurement unit and Anathomic Therapeautic Chemical (ATC) classification. The antiviral usage data was reported as antivirals for systemic usage, ATC group J05, DDD/1000person/day (DDD/1000).

## Results

The laboratory verified influenza cases between the years 2007-2010 in Turkey and in the United States are presented in table 1. Oseltamivir and zanamivir consumption quantities between the dates of 2007-2010 are presented in figure 1, and the data about oseltamivir capsule and suspension consumption are presented in figure 2. While Oseltamivir consumption was 0,016 DDD / 1000 in the year 2007, it went up to 0, 14 DDD/1000 in 2009, during pandemic period. While Zanamivir consumption in 2007 was 0,00000095 DDD/1000, it became 0,0088 DDD/1000 during pandemic period (Figure 1). While substantially increase in the capsule usage was observed during flu season, the suspension for use in children did not increase at the same pace (Figure 2).

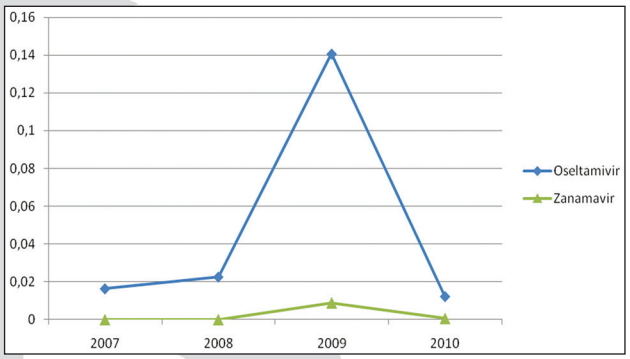


*Figure 1. Oseltamivir and zanamivir consumption as per the years*

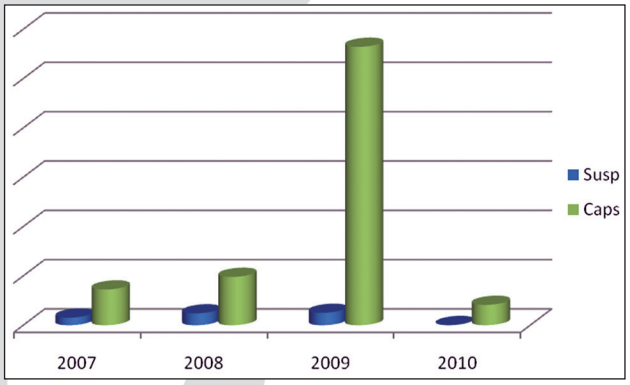


*Figure 2. Suspension and capsule usage rate over the years*

*Table 1. Influenza cases reported in USA and in Turkey by the years*

| Year | Official Flu Cases USA | Official Flu Cases Turkey (Unpublished Data, Ministry of Health, Turkey) |
|---|---|---|
| 2007 | 23.753 | 21 |
| 2008 | 39.827 | 55 |
| 2009 | 24.793 | 749 |
| 2010 | 89.585 | 142 |

## Discussion

The data of this study is one of the most important examples in examining the effects of influenza pandemic on people's and doctors' behaviours. During the pandemic period, the news in written and visual media impressed the people and caused them to use antivirals in fear for their lives, even for cases that can normally be treated merely by symptomatic treatments. Oseltamivir and although to a lesser extent zanamivir consumption peaked during the pandemic year was experienced. Oseltamivir consumption which was normally at 0.02 DDD/1000 level increased sevenfold during the pandemic period (Figure 1). We consider that this situation was a good example to the effect of the increase in drug consumption due to illness perception.

Zanamivir is used in the form of dust inhaled by the way of the mouth, by a diskhaler device. It is not recommended in the treatment and prophylaxis of the patients who have asthma or chronic obstructive pulmonary disease because of severe bronchospasm risk [8]. The difficulty in using zanamivir in the form as it is offered to the market and not being recommended for the patients having chronic obstructive pulmonary diseases, may cause this drug become the less preferred one. Diggory et all. reported in their research that a lot of elderly patients were unable to use the diskhaler properly [9]. Also, the difference in the recognition of the zanamivir as compared with oseltamivir among doctors may influence its consumption.

For the antiviral resistance, interaction of viruses with the drug is important. Travelling, low dose and short-term usage of drugs are significant for drug resistance. In parallel with the increase in the usage of antiviral drugs, antiviral resistance also has been started to increase. When oseltamivir resistance in the influenza A (H1N1) strains was 0.7% in 2006-2007 season, it was increased to 10.9 % in 2007-2008 season, and to 99.3% in 2008-2009 season[10,11,12]. During the 2009-2010 season in the United States, oseltamivir-resistant 2009 H1N1 influenza A strains were detected in 64 patients. It was ascertained that 52 of these patients (81,3%) had exposure to oseltamivir either for treatment or for chemoprophylaxis purposes [13]. However, no resistance to oseltamivir was identified in the other influenza A (H3N2) and influenza B (H1N1) strains detected in the years of 2007-2010. All tested viruses, including oseltamivir – resistant influenza A (H1N1) strains were sensitive to Zanamivir, in these years [10,11,12,13]. European Center for Disease Prevention and Control (ECDC) has reported a total of 53,658 laboratory verified influenza cases from its member countries. It was reported that; oseltamivir resistance was detected in 98.5% of influenza A (H1N1) strains that tested for resistance, and not detected in the influenza A and B strains, except H1N1 strains. In this report it was stated that all strains were sensitive to zanamivir [14].

According to our results obtained in this study, the confirmed flu cases were considerably high in Turkey during the flu pandemic, in 2009 (table 1). The continuous news on the media about flu, icreased the flu fear among people, and applications to doctor due to flu has increased. This also augmented the laboratory needs for diagnosis of flu among doctors and increased the number of antiviral prescribing for flu.

Oseltamivir consumption in Turkey during pandemic period, nearly increased sevenfold as compared to previous years, on DDD base. We attribute the increase of flu-related antiviral consumption during pandemic period might associate public sensitivity and the fear of death related with pandemic.

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## References

1. *World Health Organization (WHO). Influenza (Seasonal). [Cited in 2011]. Available from: http: //www.who.int/mediacentre/factsheets/fs211/en/index.html*

2. *World Health Organization (WHO). Influenza. [Cited in 2011]. Available from: http: //www.who.int/topics/influenza/en/*

3. *World Health Organization (WHO). WHO guidelines for pharmacological management of pandemic influenza A(H1N1) 2009 and other influenza viruses. Revised February 2010. [Cited in 2011]. Available at: http: //www.who.int/csr/resources/publications/swineflu/h1n1_use_antivirals_20090820/en/index.html*

4. *Smith JR, Rayner CR, Donner B, Wollenhaupt M, Klumpp K, Dutkowski R. Oseltamivir in seasonal, pandemic, and avian influenza: a comprehensive review of 10-years clinical experience. Adv Ther. 2011 Nov; 28(11): 927-59.*

5. *Oqbru O. Zanamivir, Relenza. [Cited in 2011]. Available from: http: //www.medicinenet.com/zanamivir/article.htm*

6. *T.C. Sağlık Bakanlığı. Grip, Virolojik sürveyans verileri. [Cited in 2011]. Available from: http: //www.grip.gov.tr/index.php?option=com_content&view=article&id=801: hafta86&catid=136: duenyada-son-durum&Itemid=523*

7. *Intercontinental Medical Statistics (IMS) Health- Turkey Office. Relenza, Highlights of prescribing information. [Cited in 2011]. Available from: http: //us.gsk.com/products/assets/us_relenza.pdf*

8. *Diggory P, Fernandez C, Humphrey H, Jones V, Murphy M. Comparison of elderly people's technique in using two dry powder inhalers to deliver zanamivir: randomised controlled trial. BMJ 2001; 322: 1-4.*

9. *Centers for Disease Control and Prevention (CDC). Update: Influenza activity--United States and worldwide, 2006-07 season, and composition of the 2007-08 influenza vaccine. MMWR Morb Mortal Wkly Rep. 2007 Aug 10; 56(31): 789-94.*

10. *Centers for Disease Control and Prevention (CDC). Influenza activity—United States and worldwide, 2007-08 season. MMWR Morb Mortal Wkly Rep. 2008 Jun 27; 57(25): 692-7.*

11. *Centers for Disease Control and Prevention (CDC). Update: influenza activity--United States, September 28, 2008-April 4, 2009, and composition of the 2009-10 influenza vaccine. MMWR Morb Mortal Wkly Rep. 2009 Apr 17; 58(14): 369-74.*

12. *Centers for Disease Control and Prevention (CDC). Update: influenza activity United States, August 30, 2009-March 27, 2010, and composition of the 2010-11influenza vaccine. MMWR Morb Mortal Wkly Rep. 2010 Apr 16; 59(14): 423-30.*

13. *ECDC Surveillance Report. Influenza surveillance in Europe 2008/09, Week 40/2008 to week 39/2009. [Cited in 2011]. Available from: http: //ecdc.europa.eu/en/publications/Publications/1005_SUR_Influenza_Europe.pdf*

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# Effects of meteorological factors on gastroesophageal varices hemorrhage in HBV-related cirrhosis

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## Abstract

**Objective:** To investigate seasonal and monthly variation of gastroesophageal variceal hemorrhage (GEVH) and associations with meteorologic factors in Liaocheng, China.

**Methods:** Admission data on 412 patients with 875 episodes of GEVH and the meteorologic data between January 2007 and December 2011 were analyzed.

**Results:** The number of episodes of GEVH showed significant seasonal and monthly variation ($p < 0.001$); specifically, bleeding was most frequent in January (n = 98) and winter (n = 279) and least frequent in July (n = 52) and summer (n = 163). The seasonal and monthly variation in GEVH had an inverse relationship with the mean temperature, rainfall, and accumulated temperature ($p < 0.05$), and a parallel relationship with the mean atmospheric pressure ($p < 0.001$). The meteorologic factors associated with the daily onset of GEVH, adjusted for Child-Pugh grade, included maximum atmospheric pressure ($p < 0.001$, OR = 1.014), maximum temperature ($p = 0.004$, OR = 0.991), and range of daily atmospheric pressure ($p = 0.019$, OR = 1.005) on the day of onset.

**Conclusions:** GEVH in Liaocheng has a clear seasonal and monthly variation; the meteorologic-related risk factors may include atmospheric pressure, temperature, and range of daily atmospheric pressure.

**Key words:** Gastroesophageal variceal hemorrhage, seasonal variation, meteorologic factors.

## Introduction

Approximately 90% of patients with cirrhosis will develop gastroesophageal varices within 10 years. Gastroesophageal variceal hemorrhage (GEVH) is a devastating complication of cirrhosis with a mortality rate of 25%-50% [1]. If patients survive a variceal bleed, there is approximately a 70% risk that they will have a further bleed within the following 2 years. Therefore, identifying risk factors of GEVH is important for prevention. Constipation, vomiting, severe coughing, and excessive consumption of alcohol may precipitate rupture of esophageal varices (EV) [2]. Some studies have suggested the existence of seasonal variation in the incidence of upper gastrointestinal bleeding; however, the role of meteorologic factors has not been elucidated [3-5]. Several investigators have also suggested a possible contribution to the onset of GEVH by meteorological factors, but the results are controversial [4, 6-8]. To evaluate the association between the onset of GEVH and meteorologic factors, it is likely to be more meaningful to analyze the short- and long-term effects of meteorologic factors, thus we designed the present study using seasonal, monthly, and daily data for meteorologic factors and the onset of GEVH to assess the seasonal variation of GEVH and correlation with meteorologic factors.factors and the onset of GEVH to assess the seasonal variation of GEVH and correlation with meteorologic factors.

## Materials and methods

### *Study population*

We conducted the study in Liaocheng, China, which has a population of approximately 1 million. Liaocheng has a land area of 1254 km$^2$, is located between 35°47' N and 115°16′ E, and has a temperate monsoon climate. Liaocheng People's Hospital is a tertiary hospital covering the entire area and is the largest hospital of the city. The majority of patients with upper gastrointestinal bleeding are admitted to Liaocheng People's Hospital.

This is a retrospective study. We reviewed all the records of patients with GEVH between 1 January 2007 and 31 December 2011 in our hospital's electronic medical record system based on the ICD-10 codes, K74.601 (HBV-related cirrhosis of liver), I85.001 (esophageal varices with bleeding) or K74.609 + (cirrhosis of liver + esophageal varices with bleeding). Patients whose bleeding was from varices were included. Variceal bleeding was diagnosed when varices were bleeding actively or showed stigmata of recent bleeding and/or if fresh blood was observed in the stomach and varices were the only potential source of bleeding. When more than one lesion was noted on endoscopy, bleeding was attributed to the lesion in which the endoscopic features made it the most likely cause of bleeding. Patients without a gastroendoscopic examination were excluded.

***Data collection***

All of the meteorologic data were obtained from the Liaocheng Meteorological Bureau. The meteorologic factors included daily atmospheric pressure (hPa, mean, maximum, minimum), ambient temperature (0.1°C, mean, maximum, minimum), relative humidity (%, mean, minimum), wind speed (0.1 m/s, mean), rainfall (0.1 mm), and hours of sunshine (0.1 h). Data for each variable were obtained every year (2007-2011). The range of daily atmospheric pressure was the difference between the maximum and minimum atmospheric pressure, and the range of daily temperature was the difference between the maximum and minimum temperature. The mean monthly and seasonal values were calculated using daily data on atmospheric pressure, ambient temperature, relative humidity, wind speed, rainfall, hours of sunshine, and accumulated temperature.

GEVH was recorded each month for 5 years (2007-2011), and monthly differences were evaluated. The 12 months of the year were divided into 4 seasonal periods: spring (March through May), summer (June through August), autumn (September through November), and winter (December through February), and seasonal differences were also evaluated.

***Statistical analyses***

The data are shown as the mean ± standard deviation. The seasonal and monthly variation of GEVH episodes was analyzed by non-parametric testing. The Spearman rank correlation coefficient was used to determine the correlations of seasonality and monthly GEVH and meteorologic factors. The correlations between daily GEVH and meteorologic factors were evaluated by logistic regression analysis. The Statistical Package for Social Sciences (version 17.0; SPSS, Inc., Chicago, IL, USA) was used. A value of $p < 0.05$ was considered significant.

## Results

Four hundred twelve patients with 875 episodes of GEVH were enrolled in the study. The patient ages ranged between 16 and 75 years, with a mean of 51 years. Five hundred eighteen GEVH episodes occurred in males (59.2%) and 357 episodes of GEVH were females (40.8%). One hundred one patients had 2 episodes of GEVH, 79 had 3 episodes, and 57 had > 3 episodes. Two hundred eighty-six patients were ever-treated with propranolol or/and esophageal variceal ligation to prevent re-bleeding, and 116 patients were ever-treated with splenectomy combined with pericardial blood vessel disarticulation. The clinical and demographic characteristics of the patients enrolled in the study are shown in Table 1.

***Seasonal variation of GEVH and meteorologic factors***

With respect to bleeding episodes by season (Figure 1), GEVH was least frequent in summer (163 [18.6%]) and highest in winter (279 [31.9%]). The number of bleeding episodes showed significant seasonal variation by non-parametric testing ($\chi^2$ = 749.449, $p < 0.001$). Based on Spearman rank correlation analysis (Table 2), the findings showed an inverse relationship between the number of episodes of GEVH and ambient temperature (R = -0.683, $p$ = 0.001), rainfall (R = -0.674, $p$ = 0.001), hours of sunshine (R = -0.612, $p$ = 0.004), and accumulated temperature (R = -0.679, $p$ = 0.001), In contrast, the relationship between the number of episodes of GEVH and atmospheric pressure was parallel (R = 0.772, $p < 0.001$). There was no correlation between the number of episodes of GEVH and humidity and wind speed ($p = 0.093$, $p = 0.709$, respectively).

*Table 1. Clinical and demographic characteristics of the patients enrolled in the study*

| Varibles | N = 875 |
|---|---|
| Gender | |
| male | 518 (59.2%) |
| female | 357 (40.8%) |
| Age (years) | |
| ≤ 18 | 31 (3.5%) |
| 19-59 | 596 (68.1%) |
| ≥ 60 | 248 (28.3%) |
| Child-Pugh grade | |
| A | 65 (7.4%) |
| B | 492 (56.2%) |
| C | 318 (36.3%) |
| Esophageal varices | |
| small ( ≤ 5 mm) | 106 (12.1%) |
| large ( > 5 mm) | 769 (87.9%) |
| 6-week mortality | 58 (6.6%) |
| Blood transfusion (U) | 1.5 ± 2.6 |
| Hemoglobin (g/dL) | 7.2 ± 2.7 |
| Platelet (*$10^9$) | 132 ± 105 |
| INR | 1.32 ± 0.27 |
| Albumin (g/L) | 28.7 ± 6.4 |
| Bilirubin (mmol/L) | 22.9 ± 31.1 |
| Creatinine (umol/L) | 73.0 ± 26.7 |
| Na (mmol/L) | 128 ± 15 |

### *Monthly variation of GEVH and meteorologic factors*

Figure 2 presents the number of episodes of GVEH in greater detail. The number of episodes of GVEH was fewest in July (52 [5.9%]) and highest in January (98 [11.2%]); the number of episodes of GVEH was also elevated in December (93 [10.6%]) and February (88 [10.1%]). There were significant differences in the number of episodes of GVEH among the months by non-parametric testing ($\chi^2 = 48.258$, $p < 0.001$).

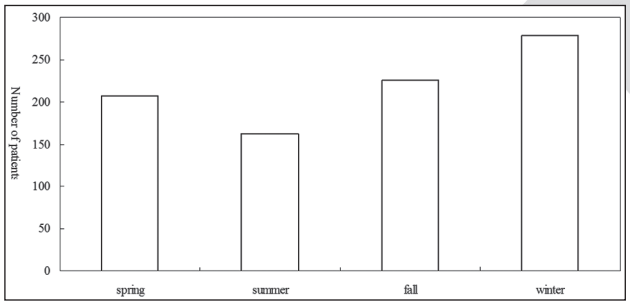


*Figure 1. The number of gastroesophageal variceal hemorrhage episodes by season between 2007 and 2011*

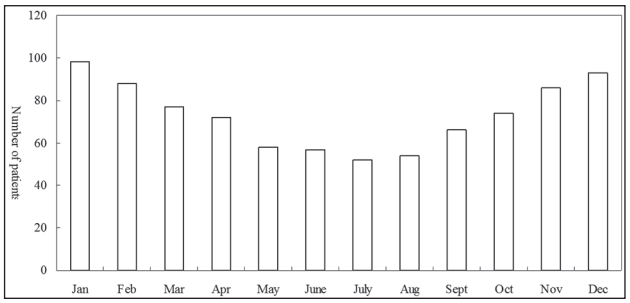


*Figure 2. The number of gastroesophageal variceal hemorrhage episodes by month between 2007 and 2011*

Table 3 shows the monthly occurrence of patients with GVEH and the mean monthly values of meteorologic data. The highest atmospheric pressure was 10235 hPa in January and the lowest atmospheric pressure was 9994 hPa in July. The highest temperature was 25.6 °C in July and the lowest temperature was -2.4 °C in January. The highest rainfall was 124.4 mm in July and the lowest rainfall was 3.6 mm in December. The highest accumulated temperature was 798.3 °C in July and the lowest accumulated temperature was 14.0 °C in January. Based on Spearman rank correlation analysis, the higher occurrence of GEVH was correlated with higher atmospheric pressure, lower temperature, less rainfall, and lower accumulated temperature (R = 0.772, R = -0.839, R = -0.726, R = -0.843, respectively, $p < 0.001$), but not correlated with relative humidity, wind speed, and hours of sunshine ($p > 0.05$).

### *Daily onset of GEVH and meteorologic factors*

Multivariate logistic regression analysis was performed to evaluate the association between the incidence of GEVH and daily meteorologic factors adjusted for Child-Pugh grade (Table 4), and demonstrated that daily maximum atmospheric pressure, maximum temperature, and range of atmospheric pressure to be significantly associated with GEVH ($\beta = 0.014$, $p < 0.001$; $\beta = -0.009$, $p = 0.004$; $\beta = 0.005$, $p = 0.019$, respectively; OR = 1.014, 0.991, 1.005, respectively). However, no significant association was demonstrated between other meteorologic factors and the incidence of GEVH ($p > 0.05$).

*Table 2. The number of episodes of gastroesophageal variceal hemorrhage and average seasonal values for meteorologic factors between 2007 and 2011*

| Season | N | Atmospheric pressure (hPa) | Ambient temperature (0.1°C) | Relative humidity (%) | Wind speed (0.1m/s) | Rainfall (0.1mm) | Hours of sunshine (0.1h) | Accumulated temperature (0.1°C) |
|---|---|---|---|---|---|---|---|---|
| Spring | 207 | 10111 | 144 | 61 | 23 | 436 | 65 | 4401 |
| Summer | 163 | 10011 | 253 | 75 | 15 | 966 | 51 | 7743 |
| Autumn | 226 | 10160 | 136 | 70 | 15 | 289 | 49 | 4181 |
| Winter | 279 | 10214 | 3 | 60 | 20 | 59 | 43 | 514 |

*Table 3. The number of gastroesophageal variceal hemorrhage episodes and average monthly values for meteorologic factors between 2007 and 2011*

| Month | N | Atmospheric pressure (hPa) | Ambient temperature (0.1°C) | Relative humidity (%) | Wind speed (0.1m/s) | Rainfall (0.1mm) | Hours of sunshine (0.1h) | Accumulated temperature (0.1°C) |
|---|---|---|---|---|---|---|---|---|
| Jan | 98 | 10235 | -24 | 57 | 20 | 52 | 45 | 140 |
| Feb | 88 | 10187 | 30 | 63 | 21 | 88 | 42 | 974 |
| Mar | 77 | 10158 | 80 | 56 | 26 | 197 | 56 | 2516 |
| Apr | 72 | 10117 | 146 | 61 | 22 | 380 | 70 | 4306 |
| May | 58 | 10056 | 206 | 66 | 21 | 731 | 71 | 6380 |
| June | 57 | 10006 | 251 | 68 | 16 | 784 | 61 | 7483 |
| July | 52 | 9994 | 256 | 78 | 14 | 1244 | 45 | 7983 |
| Aug | 54 | 10032 | 252 | 79 | 14 | 869 | 48 | 7764 |
| Sept | 66 | 10106 | 204 | 72 | 13 | 568 | 50 | 6147 |
| Oct | 74 | 10158 | 148 | 73 | 15 | 235 | 47 | 4585 |
| Nov | 86 | 10216 | 58 | 66 | 18 | 64 | 52 | 1811 |
| Dec | 93 | 10219 | 3 | 61 | 18 | 36 | 43 | 428 |

*Table 4. Multivariate logistic regression analysis for episodes of gastroesophageal variceal hemorrhage and daily meteorologic factors*

| Variables | B | SE | Wals | p value | OR | 95% confidence intervals | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower | Upper |
| Maximum atmospheric pressure | 0.014 | 0.004 | 15.049 | 0.000 | 1.014 | 1.007 | 1.021 |
| Maximum temperature | -0.009 | 0.003 | 8.095 | 0.004 | 0.991 | 0.984 | 0.997 |
| Range of atmospheric pressure | 0.005 | 0.002 | 5.458 | 0.019 | 1.005 | 1.001 | 1.010 |
| Constant | -113.961 | 13.638 | 69.825 | 0.000 | 0.000 | - | - |

## Discussion

Variceal bleeding is one of the major complications of liver cirrhosis, and is associated with significant mortality and morbidity [9]. In addition to variceal size, red wale marks on varices and advanced liver disease (Child class B or C) identify patients at high risk for variceal hemorrhage [10]. Empirical evidence supports the clinically observed relationship between the effects of weather and climatic conditions on morbidity and mortality in cases of GVEH [11, 12]. This retrospective study demonstrated the expected significant seasonal and monthly variations of GEVH during the study period, which may be attributed to the variable effects of meteorologic factors. The higher occurrence of variceal bleeding was correlated with lower temperature, higher atmospheric pressure, less rainfall, and lower accumulated temperature. Seasonal patterns between mortality and diseases may be multivariate rather than univariate; it is the whole air mass that is over us at any given time that affects us, thus we need to take into account all of these factors rather than one or several.

There are a few reports that have discussed the existence of a seasonal pattern in the incidence of GEVH and meteorologic factors and the relationship with episodes of bleeding. Yen et al. [11] reported that the incidence of bleeding from varices increased in February and decreased in July. Tahri et al. [7] reported that the seasonal distribution of variceal bleeding episodes had the highest percentage during winter; the incidence of variceal bleeding is associated with the daily mean temperature, rainfall, and stormy weather, but not associated with mean atmospheric pressure, daily hours of sunshine, nebulosity, direction and velocity of wind, and mean humidity. Sezgin et al. [4] showed that EV bleeding is most frequent in March and spring, and least frequent in September and autumn. Our findings do not agree with those of López-Cepero et al.[6] and Nomura et al.[5]; specifically, the results of those studies did not support the existence of a seasonal or monthly variation in the incidence of gastroesophageal variceal bleeding. López-Cepero et al. [6] reported that the role of climatic factors is not involved in episodes of bleeding. The difference may be attributed to different populations, races, regions, and living habits; regions with temperate monsoon climates may be the key factors. Our study had more patients and a longer time scale, thus accounting for the more evident association between GEVH and seasonal variation. A retrospective study of 13,514 hospitalizations and deaths from variceal bleeding (caused by portal hypertension) during 1987-1996 in France likewise revealed that mortality from such causes significantly peaked in January and was at the lowest level in July [8]. The researchers and our study showed that awareness of such seasonal patterns could allow practitioners to reduce mortality via early diagnosis and treatment.

When we consider how weather affects our health, we should take into account both short- and long-term weather effects. The results of the current study indicated that the variation in temperature and atmospheric pressure are attributed to both seasonal and monthly variations and daily onset of GEVH, while rainfall did not contribute to the daily onset of GEVH, and a high range in daily atmospheric pressure at the onset day of GEVH has been identified as a possible risk factor, which is never reported.

Why is GEVH more frequent in the winter and the months of December, January, and February? Although the answer is far from being conclusive, several factors are thought to play a role in GEVH seasonality and monthly variation; the indirect effect of air conditions on portal blood pressure through neurohumoral mediators seems more likely [12]. Wittert et al. [13] demonstrated that acute cold stress inhibits the release of vasopressin from the posterior pituitary in both rats and humans. The fall in serum vasopressin levels in the cold weather may explain the increased frequency of GEVH in the winter.

Other risk factors for variceal bleeding, such as seasonal differences in holiday drinks, a busy farming season, and NSAID usage, may also be related to the occurrence of variceal bleeding during seasonal changes. Silm et al. [13] suggested that there is a clear seasonal variability in alcohol consumption, with the highest wine volume consumed in December, and that the seasonal variability in alcohol consumption is influenced by meteorologic factors, such as temperature and humidity. It is plausible that seasonal differences in alcohol consumption might contribute to the seasonality of GEVH. It is possible, in fact, that susceptibility to different factors, such as NSAIDs, alcohol consumption, and meteorologic factors may have an impact on the different seasonal peaks of GEVH. Moreover, very little is known about the possible influence of biological rhythms on the occurrence of acute EV bleeding. Although this study does not provide insight into pathogenic factors, the possible demonstration of seasonal and monthly variation and meteorologic factors on GEVH may prompt further research to better understand seasonality and guide appropriate prophylaxis and management of GEVH.

In conclusion, there are seasonal and monthly variations in GEVH, which may be related to seasonal and monthly changes in meteorologic factors. Higher atmospheric pressure, lower temperature, less rainfall, and lower accumulated temperature may contribute to the occurrence of GEVH in our city, especially a large change in atmospheric pressure on the day of onset of GEVH. Although the reason for these relationships is not clear, meteorologic factors may play an important role in variceal bleeding. Further studies exploring the pathophysiology involved in the effect of meteorologic factors on GEVH are required.

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**Reference**

1. *Garcia-Tsao G, Sanyal AJ, Grace ND, et al. Prevention and management of gastroesophageal varices and variceal hemorrhage in cirrhosis. Hepatology 2007;46: 922-938.*

2. *Liao W-C, Hou M-C, Chang C-J, et al. Potential Precipitating Factors of Esophageal Variceal Bleeding: A Case–Control Study. Am J Gastroenterol 2010;106: 96-103.*

3. *Du T, Lewin MR, Wang H, et al. Circadian and seasonal rhythms of acute upper gastrointestinal bleeding in Beijing. Emerg Med J 2010;27: 504-507.*

4. *Sezgin O, Altintas E, Tombak A. Effects of seasonal variations on acute upper gastrointestinal bleeding and its etiology. Turk J Gastroenterol 2007;18: 172-176.*

5. *Nomura T, Ohkusa T, Araki A, et al. Influence of climatic factors in the incidence of upper gastrointestinal bleeding. J Gastroenterol Hepatol 2001;16: 619-623.*

6. *Lopez-Cepero JM, Lopez-Silva ME, Amaya-Vidal A, et al. Influence of climatic factors on the incidence of upper gastrointestinal bleeding. Gastroenterol Hepatol 2005;28: 540-545.*

7. *Tahri N, Amouri A, Fekih H, et al. Meteorologic conditions and esophageal varices rupture. Ann Med Interne (Paris) 2003;154: 509-514.*

8. *Boulay F, Berthier F, Dahan MDC, et al. Seasonal variations in variceal bleeding mortality and hospitalization in France. Am J Gastroenterol 2001;96: 1881-1887.*

9. *Sarin SK, Kumar A, Angus PW, et al. Diagnosis and management of acute variceal bleeding: Asian Pacific Association for Study of the Liver recommendations. Hepatol Int 2011;5: 607-624.*

10. *Garcia-Tsao G, Bosch J. Management of varices and variceal hemorrhage in cirrhosis. N Engl J Med 2010;362: 823-832.*

11. *Yen FS, Wu JC, Wang LM, et al. Seasonal variation in the incidence of peptic ulcer and esophageal variceal bleeding in Taiwan. Zhonghua Yi Xue Za Zhi (Taipei) 1996;57: 22-27.*

12. *Soylu AR, Oksuzoglu G, Tatar G, et al. The effect of seasons on variceal bleeding in patients with cirrhosis. Am J Gastroenterol 1996;91: 823-824.*

13. *Silm S, Ahas R. Seasonality of alcohol-related phenomena in Estonia. Int J Biometeorol 2005;49: 215-223.*

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# The effect of serum folate on plasma homocysteine, lipid profile, and occurrence of premature coronary heart disease

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**Abstract**

Folic acid can play a protective role in coronary heart disease. However, whether this is due to a reduction in homocysteine or some other effect remains uncertain. We aimed at assessing the effect of serum folate on plasma homocysteine and lipd profile in premature coronary heart disease. A retrospective case control study with 51 incident cases of acute coronary syndrome, and 52 healthy controls, aged less than 55years. Compared to controls, participants with acute coronary syndrome had a significantly higher waist circumference (98. 0 ±11. 7 cm in cases versus 93. 9 ±8. 4cm in controls), triglycerides (2. 1 ±1. 1 mmol/l in cases versus 1. 5 ±0. 8 mmol/l in controls), and a significantly lower high density lipoprotein (0. 9 ±0. 2mmol/l in cases versus 1. 1 ±0. 3 mmol/l in controls). Total homocysteine and serum folate concentrations showed no significant difference between cases and controls. When adjusted, Odd's ratio for high density lipoprotein was a significant strongly protective factor (*exp B*= 1327. 6**). Serum folate adjusted Odd's ratio showed a protective effect among smokers (*exp B*= 0. 197*). In the whole sample, plasma total homocysteine, showed graded inverse associations across folate quintiles. The existing data support that low-dose folate treatment (achieved by fortification) may reduce premature cardiovascular risk, particularly in young smokers. Our findings add uncertainty to previous conclusions that total homocysteine is a major, independent, causative factor for coronary heart disease.

**Key words:** Folate, total homocysteine, smoking, coronary heart disease, Asians.

## Introduction

Asians show increased risk for atherosclerosis and have high mortality rates owing to coronary heart disease (CHD). (1)

Studies suggested that Asians are at increased risk of myocardial infarction at a younger age (<40 years). (2) Premature CHD is characterized by an unfavorable lipid profile, low concentrations of HDL-C and high triglyceride levels. (3)

On the other hand, moderately elevated blood levels of homocysteine are weakly correlated with CHD risk, but causality remains uncertain. (4) Mild or moderate cases of hyperhomocysteinemia (tHcy > 15 μmol/lit), prevalent in the general population, can be the result of dietary deficiency of factors such as folic acid. (5) Other etiologies of hyperhomocysteinemia include end stage renal disease (6), hypothyroidism (7), tobacco smoking (8), and the use of medications, such as antiepileptic drugs (9), isoniazid (10), methotrexate, (11), N acetyl cysteine (12), and thiazide diuretics (13).

Studies showed that folic acid improves endothelial function (14), hence therapy with folic acid was suggested as a treatment to reduce the risk of cardiovascular events. Despite results of several completed trials in high-risk populations were disappointing, (15) (16) (17) it is not time to close the book on folic acid and cardiovascular health.

## Materials and Methods

In this study, we sought to test the effects of serum folate, plasma tHcy, and lipid profile in a retrospective case control study, on occurrence of the first

cardiac event in young and middle aged men. The study protocol was approved by the Research ethics committee of King Abdulaziz University Hospital. All participants signed a written informed consent.

***Recruitment and follow-up***

Participants were males, <55 years. Patients were incident cases of acute coronary syndrome ($n$ =51), who were recruited from the coronary care unit at the King Abdulaziz University Hospital. Clinicians were responsible to give the potential study participants (i. e. patients that meet the eligibility criteria) the first information about the study protocol.

Controls of same age and gender ($n$ =5٢) were recruited for this study by public advertisement at the same hospital, and were free from diabetes (defined as fasting blood glucose ≥ 5. 1 mmol/l or current use of antidiabetic medication ), hypertension (defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg, or current use of antihypertensive medication ), and had a BMI not more than 35.

Participants should have experienced a recent coronary acute event. To fulfill a coronary acute event criteria, the patients must have had either a documented myocardial infarction, defined by at least 2 of the following criteria (Typical chest pain, Electrocardiographic modifications related to myocardial infarction, Cardiac enzymes increase), or an acute coronary syndrome without myocardial infarction, defined by 2 of the following criteria (Typical chest pain, electrocardiographic modifications related to acute coronary syndrome, a coronary artery disease documented with an angiographic coronary stenosis over 50%, or a positive exercise test).

Were not included those with thyroid disease, congestive heart failure, patients with an end stage non-cardiovascular pathology (solid cancer, evolved dementia, leukemia) liver enzymes >2 times upper limit of the reference range, chronic renal failure (plasma level of creatinine > 200 μmol/L ), malabsorption, gastrectomy, treatment with hypolipidemic drugs for ≥3 months, intake of supplements (antioxidant, omega 3 fatty acids, vitamin B12, folic acid and N-Acetyl Cysteine ) within 6 weeks of enrollment, also intake of medications as antiepileptic drugs, methotrexate, isoniazid and thiazide diuretics.

Eligible participants were physically examined, blood pressure measurements were performed; weight and height were measured by standard procedures, and BMI calculated. Waist circumference was measured as the smallest circumference between costal margin and anterior superior iliac spine, as soon as the patient could ambulate. An expert dietitian interviewed the patients and controls about their habitual diet, to exclude vegetarians, and those following a dietetic advice for hyperlipidemia. In addition, all subjects were interviewed for detailed medical, personal, and family history. A complete blood count, FBS, TSH, liver enzymes, serum creatinine, were measured with the use of standard laboratory techniques. In addition, a 12 hour fasting plasma lipid profile (total cholesterol, triglycerides (TG), LDL, HDL), as well as serum folate concentration were taken. Sample for fasting plaasma tHcy was taken, 24–36 h after onset of acute myocardial infarction, held on ice.

To test study hypotheses, we used statistical package for social sciences (SPSS) version16 (SPSS Inc, Chicago). [18] We first used independent sample t test to compute geometric mean values of study variables for CHD cases versus the controls: age, BMI, waist circumference, plasma lipid profile (total cholesterol, TG, LDL, HDL), fasting tHcy, as well as serum folate concentration. We then computed weighted correlations among risk factors of acute coronary syndrome, among which serum folate, tHcy, and lipid profile. We estimated odd's ratio and 95% CIs of CHD in relation to categories of study variables using binary logistic regression for age, smoking (never, former, current), history of hypertension or diabetes (no and yes), HDL, and serum folate.

Finally, to determine the relation of serum folate with tHcy, some of which may be confounders in this analysis, we categorized the whole sample into fifths based on quintile cut points and used ANCOVA to calculate means. We used 4 sets of covariates. Model 1 was adjusted for age; model 2, for the covariates in model 1 plus smoking, BMI, and systolic blood pressure; model 3, for the covariates in model 2 plus serum LDL and HDL cholesterol.

## Results

### *Difference in means between cases and controls*

Compared with controls, participants with acute coronary syndrome tended to have a significantly higher waist circumference (98. 0 ±11. 7 cm in CHD cases versus 93. 9 ±8. 4cm in controls), TG (2. 1 ±1. 1 mmol/l in CHD cases versus 1. 5 ±0. 8 mmol/l in controls), and a significantly lower HDL (0. 9 ±0. 2mmol/l in CHD cases versus 1. 1 ±0. 3 mmol/l in controls) (Table 1). tHcy and serum folate concentrations showed no significant difference between cases and controls.

*Table 1. Comparison between cases and controls as regards anthropometrical and biochemical data*

| Variable | | Range | Mean ±SD | t | df | p |
|---|---|---|---|---|---|---|
| **BMI** | case | 18. 4-36. 5 | 26. 9 ±4. 0 | -0. 47 | 100 | 0. 64 |
| | control | 22-35 | 27. 6 ±3. 5 | | | |
| **Waist circumference** | case | 81-135 | 98. 0 ±11. 7 | 2. 17 | 94 | 0. 03* |
| | control | 74-115 | 93. 9 ±8. 4 | | | |
| **Serum TC** | case | 3-9 | 5 ±1. 3 | -0. 11 | 101 | 0. 91 |
| | control | 2. 8-8. 2 | 5 ±1. 0 | | | |
| **Serum TG** | case | 0. 8-4. 96 | 2. 1 ±1. 1 | 3. 44 | 92. 4 | 0. 00*** |
| | control | 0. 7-4. 36 | 1. 5 ±0. 8 | | | |
| **Serum LDL** | case | 2- 6. 1 | 3. 3 ±0. 94 | -0. 48 | 98 | 0. 63 |
| | control | 1. 3-5. 7 | 3. 4 ±0. 91 | | | |
| **Serum HDL** | case | 0. 5-1. 5 | 0. 9 ±0. 2 | -6. 01 | 98 | 0. 00*** |
| | control | 0. 7-2 | 1. 1 ±0. 3 | | | |
| **Serum Hcy** | case | 5. 4-20 | 10. 6 ±3. 1 | -0. 41 | 98 | 0. 68 |
| | control | 7-20 | 11. 3 ±3. 1 | | | |
| **Serum folate** | case | 5. 5-45. 4 | 20. 1 ±7. 8 | -0. 93 | 98 | 0. 35 |
| | control | 6. 6-41. 1 | 21 ±7. 3 | | | |

*WC in cm, Serum TC, S TG, SHDL, SLDL, tHcy, S folate, in mmol/L.*
**t is significant at the 0. 05 level (two-tailed).*
***t is significant at the 0. 01 level (two-tailed).*
****t is significant at the 0. 001 level (two-tailed).*

*Table 2. Correlation between different risk factors of CHD*

| | Age | BMI | WC | TG | LDL | HDL | t Hcy | S folate | smoking | FH | DM | HT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Age** | 1. 00 | -0.05 | 0.02 | 0.15 | -0.12 | -0.28*** | 0.25* | 0.07 | 0.127 | 0.11 | 0.11 | 0.32*** |
| **BMI** | -0.05 | 1.00 | 0.68** | 0.13 | 0.013 | 0.19 | 0.08 | -0.01- | -0.23* | -0.03 | 0.06 | 0.04 |
| **WC** | 0.02 | 0.68** | 1.00 | 0.04 | 0.02 | 0.03 | 0.13 | -0.10- | -0.18 | -0.01 | 0.12 | 0.15 |
| **TG** | 0.15 | 0.13 | 0.04 | 1.00 | 0.35** | -0.39** | 0.01 | 0.06 | 0.08 | 0.3** | 0.19 | 0.39*** |
| **LDL** | -0.12 | 0.013 | 0.02 | 0.36** | 1.00 | 0.16 | 0.10 | -0.01- | 0.16 | 0.01 | -0.10 | 0.02 |
| **HDL** | -0.28* | 0.19 | 0.03 | -0.39** | 0.16 | 1.00 | -0.07- | 0.06 | -0.24* | -0.2 | -0.23* | -.22* |
| **tHcy** | 0.25* | 0.08 | 0.13 | 0.01 | 0.10 | -0.07- | 1.00 | -0.31** | 0.10 | -0.15 | -0.09 | -0.01 |
| **S folate** | 0.07 | -0.01 | -0.10 | 0.06 | -0.01 | 0.06 | -0.31** | 1.00 | -0.12 | 0.01 | -0.11 | 0.02 |
| **smoking** | 0.13 | -0.23* | -0.18 | 0.08 | 0.16 | -0.24* | 0.10 | -0.12- | 1.00 | 0.13 | 0.06 | 0.05 |
| **FH** | 0.11 | -0.03 | -0.01 | 0.3** | 0.02 | -0.2 | -0.15- | 0.01 | 0.13 | 1.00 | 0.12 | 0.25** |
| **DM** | 0.11 | 0.06 | 0.12 | 0.19 | -0.10 | -0.23* | -0.09- | -0.11- | 0.06 | 0.12 | 1.00 | 0.27** |
| **HT** | 0.32*** | 0.04 | 0.15 | 0.39*** | 0.02 | -0.22* | -0.01 | 0.02 | 0.05 | 0.25** | 0.27** | 1.00 |

*S= serum, WC= waist circumference, HT= hypertension, FH= Family history*
*Age in years, WC in cm, S TG, SHDL, SLDL, tHcy, S folate, in mmol/L.*
*Values are Spearman's r correlation coefficients. All reported P values are two-tailed.*
**Correlation is significant at the 0. 05 level (two-tailed).*
***Correlation is significant at the 0. 01 level (two-tailed).*
****Correlation is significant at the 0. 001 level (two-tailed).*

***Correlates of acute coronary syndrome, and its risk factors***

Correlations among risk factors showed that older individuals tended to have lower S HDL and higher tHcy, and be hypertensive (r= -0. 28**, 0. 25*, and 0. 32*** respectively). On the other hand, BMI correlated with waist circumference and smoking as r = 0. 68**, and r = - 0. 23* respectively.

Regarding correlations among serum lipids, Serum TG correlated with LDL, HDL, as r= 0. 35**, and r= - 0. 39** respectively. Moreover, Serum TG correlated with family history (r= 0. 3**), Serum HDL correlated negatively with age r= - 0. 28**, smoking r= - 0. 24*, diabetes r= - 0. 23*, and hypertension r= - 0. 22*. The weighted correlations of hypertension with age, S HDL, Serum TG, family history, and DM were 0. 32***, r= -0. 22*, r= 0. 387***, r= 0. 25**, r= 0. 27** respectively.

Regarding tHcy, it correlated positively with age r= 0. 25*, and negatively with serum folate r= - 0. 311** (Table 2)

The weighted correlations of CHD with TG, SHDL, smoking, positive family history, diabetes, and hypertension, *r*= 0. 36***, *r*= - 0. 57***, *r*= 0. 29**, *r*= 0. 33***, *r*= 0. 45***, and *r*= 0. 51*** respectively. (Table 3)

*Table 3. Correlation between incidence of CHD, and different predictors*

| Variable | Incidence of CHD |
|---|---|
| BMI | -0. 05 |
| Waist circumference | 0. 16 |
| Serum TG | . 36*** |
| Serum LDL | -0. 08 |
| Serum HDL | -0. 57*** |
| Serum tHcy | -0. 06 |
| Serum folate | -0. 14 |
| smoking | 0. 29** |
| Family history | 0. 33*** |
| DM | 0. 45*** |
| hypertension | 0. 51*** |

*WC in cm, Serum TC, S TG, SHDL, SLDL, tHcy, S folate, in mmol/L.*
*Values are Spearman's r correlation coefficients.*
*All reported P values are two-tailed.*
***Correlation is significant at the 0. 01 level (two-tailed).*
****Correlation is significant at the 0. 001 level (two-tailed).*

When adjusted, Odd's ratio for HDL was a significant strongly protective factor (*exp B*= 1328, p 0. 003**); whereas smoking showed a stronger positive association with CHD (*exp B*= 0. 197, p= 0. 026*), than hypertension (*exp B*= 0. 03 p= 0. 001***) among the whole sample. (Table 4).

Serum folate adjusted Odd's ratio showed a modest protective non significant effect on risk of CHD (*exp B*= 0. 97, p= 0. 55); but showed a significant relatively reduced risk of CHD among current smokers versus non smokers. (the odd's ratio of smoking, before adjustment for serum folate was (*exp B*= 0. 29, p= 0. 004**), and after adjustment was (*exp B*= 0. 2, p= 0. 03*)). (Table 5)

Among the whole sample, plasma tHcy, showed graded inverse associations across folate quintiles (Table 5). The tHcy decreased across folate quintiles and was especially low (9. 65 (0. 69), and 10. 2 (0. 67)) in the two highest quintiles of serum folate, though statistically insignificant when adjusted for age, smoking, BMI, and systolic blood pressure, serum LDL, serum HDL.

## Discussion

Recently, it has been suggested that tHcy is a marker, rather than a cause of vascular disease. [16] Elevated tHcy may react multiplicatively with other risk factors of CHD in high risk patients. [19] An interesting finding is that young patients suffering from inborn error of homocysteine metabolism do not suffer from coronary events. [20]

In the present study, we excluded cases with previous coronary events; moreover diabetic patients constituted only 17 % of our whole sample, while hypertensive ones were only 26%. This might explain the fact that tHcy levels could not correlate with incidence of CHD among the whole sample. The correlation of tHcy to age, in the present study (table 2), can be explained by renal function, which is highly associated to tHcy levels; since an inverse relationship exists between the glomerular filtration rate (GFR) and plasma homocysteine level throughout the whole range of renal function. [21]

Folates act as a substrate in the remethylation of homocysteine to methionine, [22] hence the inverse association between serum folate and tHcy, though statistically insignificant when adjusted for major risk factors. (table 5) However, serum folate dd not correlate with plasma lipid profile components (table 2).

*Table 4. Multiple logistic regression analyses for selected risk factors relative to serum folate in occurrence of acute coronary syndrome among the whole sample*

| Independent variable | B | SE | p | Exp(B) | 95 % C. I. for Exp (B) | |
|---|---|---|---|---|---|---|
| | | | | | Lower | Upper |
| Age | 0. 01 | 0. 05 | 0. 84 | 1. 01 | 0. 92 | 1. 10 |
| Smoking (current to non smokers) | -1. 63 | 0. 73 | 0. 03* | 0. 2 | 0. 04 | 0. 83 |
| HTN (no, yes) | -3. 37 | 1. 02 | 0. 00*** | 0. 034 | 0. 01 | 0. 25 |
| DM (no, yes) | -21. 9 | 8215 | 1 | 0. 00 | 0. 00 | |
| HDL | 7. 19 | 2. 43 | 0. 00** | 1328 | 11. 4 | 155019 |
| Folate | -0. 03 | 0. 05 | 0. 55 | 0. 97 | 0. 89 | 1. 06 |
| Constant | -4. 84 | 3. 27 | 0. 14 | 0. 01 | | |

**Regression is significant at the 0. 05 level (two-tailed).*
*** Regression is significant at the 0. 01 level (two-tailed).*
**** Regression is significant at the 0. 001 level (two-tailed).*

*Table 5. Serum homocysteine by quintiles of serum total folate concentration among the whole sample*

| | Quintiles of serum folate concentration | | | | | *F* | *p* |
|---|---|---|---|---|---|---|---|
| | **1 (n= 19) 11. 02 (0. 69)** | **2 (n= 21) 16. 8 (0. 23)** | **3 (n= 21) 19. 7(0. 2)** | **4 (n= 19) 23. 0 (0. 32)** | **5 (n=20) 32. 1(1. 37)** | | |
| **Model 0: unadjusted S Hcy** | 12. 6 (0. 70) | 10. 9 (0. 67) | 10. 5 (0. 67) | 9. 65 (0. 69) | 10. 2 (0. 67) | 2. 68 | 0. 04* |
| **Model 1: adjusted *a* S Hcy** | 12. 7(0. 69) | 10. 9(0. 65) | 10. 4 (0. 67) | 9. 59 (0. 67) | 10. 1(0. 6) | 1. 28 | 0. 28 |
| **Model 2: adjusted *b* S Hcy** | 12. 7(0. 74) | 11. 8(0. 83) | 10. 6(0. 69) | 9. 56 (0. 69) | 10. 2(0. 69) | 1. 77 | 0. 14 |
| **Model 3: adjusted *c* S Hcy** | 12. 7 (0. 82) | 10. 8(1. 04) | 10. 8(0. 73) | 9. 43(0. 73) | 10. 2(0. 78) | 0. 73 | 0. 57 |

*Test used: one way ancova*
*Values are mean (SE)*
*a Adjusted for age*
*bAdjusted for the covariates in model 1 plus smoking, BMI, and systolic blood pressure.*
*cAdjusted for the covariates in model 2 plus serum LDL and HDL cholesterol.*
**F is significant at the 0. 05 level (two-tailed).*

Many studies have described an unfavorable lipid profile in young coronary patients[23]. In South Asians, dyslipidemia, and smoking were the important risk factors, as in the rest of the world. In addition, abdominal obesity, hypertension, and diabetes had more severe effects in South Asia. [24] This was confirmed in the present study amongst a Saudi population. (table 3)

Though addition of family history of CHD to other risk factors causes only a mild correlation ($r$= 0 0. 33***), it must be emphasized that modifiable physiological variables such as blood pressure, lipid profile, and abdominal obesity are also partially under genetic control. [24]

***Serum folate and risk of premature CHD***

A late meta analysis failed to demonstrate a clear significant benefit of folic acid supplementation on the risk of CHD. [25]However, the present study found a modest protective non significant effect of serum folate on risk of CHD *(exp B*=. 972, p= 0. 55). (Table 4).

Folic acid has a direct protective effect on vascular function in humans, independently of any

effects on plasma Hcy. Plausible mechanisms are those through intrinsic antioxidant actions [26], particularly among young age who show a less extensive atheromatous lesions and more contribution of prothrombotic and inflammatory indices. [3]

Results of folic acid supplementation can really be disappointing in CHD prevention, since the RDA for folic acid (400 mg/day) has the ability to induce the maximum benefit on vascular function. [14]

In 2002, the government of Saudi Arabia issued a mandatory act of fortifying white wheat flour with folic acid. [27] The evaluation of fortification act among adult healthy Saudi men and women proved an eradication of the folate deficiency that was due to inadequate folate intake. [27] This might explain the absent difference in means of serum folate among cases and controls (table 1). On the other hand, when adjusted for serum folate, a significant relatively reduced risk of CHD was detected among current smokers versus non smokers. (table 4) In fact, the odd's ratio of smoking, before adjustment for serum folate was (*exp B*= 0. 286, p= 0. 004**), and after adjustment was (*exp B*= 0. 197, p= 0. 026*). This result is biologically plausible since folate may protect the vascular wall from the radical damage derived from smoking. [28]

## Limitations

The authors acknowledge several limitations. First, due to the difficulty in recruiting patients with recent acute coronary syndrome and many exclusion criteria, we were not able to produce the desired sample size, which probably explains why some findings were not significant when it should be.

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## References

1. *Sadeghian S, Graili P, Salarifar M, Karimi A, Darvish S, Abbasi S. Opium consumption in men and diabetes mellitus in women are the most important risk factors of premature coronary artery disease in Iran. Int J Cardiol 2010; 141: 116-8.*

2. *Sharma M, Ganguly N. Premature Coronary Artery Disease in Indians and its Associated Risk Factors. Vasc Health Risk Manag 2005; 1: 217–25.*

3. *Pinedaa J, Marína F, Marco P, Roldán V, Valenciaa J, Ruiz-Nodara J, Sogorba F, Lip G. Premature coronary artery disease in young (age < 45) subjects: Interactions of lipid profile, thrombophilic and haemostatic markers. Int J Cardiol 2009; 136: 222-5.*

4. *Clarke R, Bennett DA, Parish S, Verhoef P, Do¨ tsch-Klerk, Lathrop M, et al. Homocysteine and Coronary Heart Disease: Metaanalysis of MTHFR Case-Control Studies, Avoiding Publication Bias. Medicine 2012; 9. Available at http: //www. ccjm. org/content/77/12/911. full. pdf. Accessed on 11/17/2012.*

5. *De Bree A, Verschuren WM, Blom HJ, Kromhout D. Lifestyle factors and plasma homocysteine concentrations in a general population Sample. Am J Epidemiol. 2001; 154: 150–4.*

6. *Jamison RL, Hartigan P, Kaufman JS, Goldfarb DS, Warren SR, Guarino PD, Gaziano JM. Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal disease: A randomized controlled trial. JAMA 2007; 298: 1163–70.*

7. *McCully KS. Homocysteine, vitamins, and vascular disease prevention. Am J Clin Nutr 2007; 86: 1563S–8S.*

8. *Kim DB, Oh Y-S, Yoo K-D, Lee J-M, Park C, Ihm S-H, Jang S, Shim B, Kim H-Y, Seung K, Rho T-H, Kim J-H. Passive smoking in never-smokers Is associated with Increased plasma Homocysteine levels. Analysis of NHANES III Data. Int Heart J 2010; 51: 183-7.*

9. *Belcastro V, Gaetano G, Italiano D, . Oteri G, Caccamo D, Pisani LR, Striano P, Striano S, Ientile R, Pisani F. Antiepileptic drugs and MTHFR polymorphisms influence hyperhomocysteinemia recurrence in epileptic patients. Epilepsia, 2007; 48: 1990–4.*

10. *Kalbhande JG, Shejpal YK, Mehare SS. Arterial Occlusion in hyperhomocysteinaemia. Bombay Hospital J 2012; 54: 120-3.*

11. *Welch GN, Loscalzo J. Homocysteine and atherothrombosis. N Engl J Med 1998; 338: 1042–50.*

12. *Pezzini A, Del Zotto E, Padovani A Homocysteine and cerebral ischemia: pathogenic and therapeutical implications. Curr Med Chem 2007; 14: 249–63.*

13. *Westphal S, Rading A, Luley C, Dierkes J. Antihypertensive treatment and homocysteine concentrations. Metabolism 2003; 52: 261–3.*

14. *Antoniades C, Antonopoulos A, Tousouli D, Marinou K, Stefanadis C. Homocysteine and coronary atherosclerosis: from folate fortification to the recent clinical trials. Eur Heart J 2009; 30: 6–15.*

15. *Toole J, Malinow M, Chambless L, Spence J, Pettigrew L, Howard V, et al. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death. The Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. JAMA 2004; 291: 565–75.*

16. *Lonn E, Held C, Arnold J, Probstfield J, McQueen M, Micks M, et al. Rationale, design and baseline characteristics of a large, simple, randomized trial of combined folic acid and vitamins B6 and B12 in high-risk patients: the Heart Outcomes Prevention Evaluation (HOPE)-2 trial. Can J Cardiol 2006; 22(1): 47-53.*

17. *Bønaa K, Njølstad I, Ueland P, Schirmer H, Tverdal A, Steigen T, et al. for the NORVIT Trial Investigators. Homocysteine lowering and cardiovascular events after acute myocardial infarction. N Engl J Med 2006; 354: 1578–88.*

18. *Fiddler L, Hecht L, Nelson EE, Nelson EN, Ross J. SPSS for Windows 16. 0: A Basic Tutorial. Social Science Research and Instruction Center. California State University. Accessed 25/8/2011. Available at http: //www. ssric. org/trd/spss16.*

19. *Rajala U, Paivansalo P, Laakso M, Pelkonen O, Koskla P, Suramo I, Keinnanen-Kiukaanniemi S. Lack of association between early atherosclerotic carotid artery wall lesions and serum level of homocysteine. The British Journal of Diabetes and Vascular Disease 2003; 3: 230-2.*

20. *De Bree A, Verschuren WM M, Kromhout D, Mennen LI, Blom HJ. Homocysteine and coronary heart disease: the importance of a distinction between low and high risk subjects. Int J Epidemiol 2002; 31: 1268-77.*

21. *Al-Attas OS, Al-Daghri NM, Appiedu GA. Fasting homocysteine levels in a cross-section of Saudi adults with type 1 diabetes mellitus. Diabetes Metab Syndr 2009; 3: 45–49*

22. *Verhaar MC, Stroes E, Rabelink TJ. Folates and Cardiovascular Disease. Arterioscler Thromb Vasc Biol 2002; 22: 6-13.*

23. *Chen L, M. Chester M, Kaski J. Clinical factors and angiographic features associated with premature coronary artery disease. Chest 1995; 108: 364–9*

24. *Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet. 2004; 364: 937–52.*

25. *Bazzano, LA, Reynolds, K, Holder, KN, et al. Effect of folic acid supplementation on risk of cardiovascular diseases: a meta-analysis of randomized controlled trials. JAMA 2006; 296: 2720–6.*

26. *Doshi S, McDowell, Moat S, Payne N, Durrant H, Lewis M, Goodfellow J. Folic acid improves endothelial function in coronary artery disease via mechanisms largely independent of homocysteine lowering. , Circulation. 2002; 105: 22-6*

27. *Al-Assaf AH, Al-Nasier AA, Al-Khalifa AS. Folate Status of Adult Saudis in Riyadh Region –Saudi Arabia. Available at http: //faculty. ksu. edu. sa/ Al-Assaf/Documents/Folate%20Status%20of%20 Adult%20Saudis%20in%20Riyadh%20Region. pdf. Accessed on 5/31/2012.*

28. *Ishihara J, Iso H, Inoue M, Iwasaki M, Okada K, Kita Y, et al. Intake of Folate, Vitamin B6 and Vitamin B12 and the Risk of CHD: The Japan Public Health Center-Based Prospective Study Cohort I. J Am Coll Nutr 2008; 27: 127–136.*

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# Carotid intima media thickness in familial mediterranean fever

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**Abstract**

**Objectives:** Familial Mediterranean Fever (FMF) is an hereditary, autoimmune, inflammatory disease characterized by recurrent attacks of fever and sterile polyserositis. Subclinical ongoing inflammation have been demonstrated during the attack free periods. Since inflammation is a well known risk factor for endothelial dysfunction and premature atherosclerosis, we aimed to investigate if carotid intima media thickness (CIMT), which is one of the earliest morphological vascular alterations of atherosclerosis, is increased in uncomplicated FMF patients.

**Patients and methods**: Thirty-eight FMF (mean age 32.8±11) patients none of which had any complications, amyloidosis or any other risk factors for atherosclerosis and 35 healthy controls (mean age 28.1±9.1) were enrolled. Erythrocyte sedimantation rate, C-reactive protein (CRP), fibrinogen, fibrin degradation products were measured in addition to routine hematologic and biochemical analysis. B-mode ultrasound was performed for the evaluation of CIMT which is averaged from several measurements made at common carotid arteries 1 cm below the bulbus.

**Results:** Median CIMT in FMF group was higher than the control group (0.50 mm[IQR: 0.24] vs 0.45mm[IQR: 0.10] p: 0.016). Most of the inflammatory parameters like C-reactive protein (CRP), fibrinogen, fibrin dimer levels were not significantly different except erythrocyte serimentation rate (ESR) which was found to be higher in FMF patients ($p<0.001$).

**Conclusion:** CIMT increases in uncomplicated FMF, without any other cardiovascular risk factor. Long term results are needed to evaluate if this finding has any clinical relevance for FMF patients.

**Key words:** Familial mediterrannean fever, Carotid intima media thickness, premature atherosclerosis, inflammation.

## Introduction

Familial Mediterranean Fever (FMF) is an hereditary, autoimmune, inflammatory disease characterized by recurrent attacks of fever and sterile polyserositis. Pleuritis, peritonitis, synovitis/arthritis are the main manifestations of the disease (1). It is caused by genetic mutations in the Mediterranean Fever gene (MEFV) mapped on chromosome 16. Most commonly affected populations are people from Mediterranean ancestry; Turks, Arabs, Armenians and Jews (2). Subclinical ongoing inflammation have been demonstrated during the attack free periods (3). Because inflammation is a well known risk factor for endothelial dysfunction and premature atherosclerosis; FMF has been under investigation regarding many forms of cardiovascular disease.

Although functional impairment in the vasculature caused by endothelial dysfunction precedes morphological alterations, Carotid intima media thickening non-invasively detected by B-mode ultrasound, is one of the earliest subclinical identifiable manifestations of early atherosclerosis (4). For an absolute CIMT increase of 0,1 mm; 10-15% increase in the future risk of myocardial infarction and 13-18% increase in the future risk of stroke have been reported (5). There are scarce reports on CIMT in FMF with contradictory results; however, FMF has not been found to be associated with increased prevalence of ischemic heart disease (6). We aimed to search if CIMT increased in a relatively young FMF population who have not yet developed amyloidosis or any other complications.

## Method

Thirty-eight FMF patients who admitted to the Gastroenterology outpatient clinic and 35 healthy controls recruited from the voluntary blood donors who admitted to the blood transfusion center

of the hospital were enrolled to the study. Local ethics committee has approved this case-control study conducted according to the recommendations of Declaration of Helsinki on Biomedical Research involving human subjects. Written informed consent was obtained from each participant. FMF was diagnosed according to Tel-Hashomer criteria (1). All of the patients were attack free. Disclusion criteria consisted of presence of coronary artery disease, hypertension, diabetes mellitus or impaired fasting glucose (fasting bloog glucose >100 mg/dl), smoking, hyperlipidemia, left ventricular ejection fraction <%50, echocardiographic wall motion abnormality, renal failure and any chronic disease other than FMF. After a detailed history and physical examination, standart laboratory methods were used for the analysis of complete blood count, biochemistry, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), fibrinogen, fibrin degradation products and urinalysis of the participants.

Evaluation of CIMT was performed under near-optimal conditions in a quite ultrasonography laboratory after 15 minutes of supine rest between 09.00AM-10.00AM. All measurements were performed by the same cardiologist blinded to the participant's clinic. High resolution B-mode ultrasonography was performed by an ultrasound device (GE Vingmed Utrasound, Horten, Norway) equipped with 7.5-10 MHz linear array transducer. Two-dimensional images of the far wall of the right and left common carotid arteries 1 cm distal to the carotid bulb were displayed. Three consecutive measurements during end-diastole were averaged for each carotid artery; the final CIMT was calculated as the average of right and left carotid arteries (7).

***Statistical Analysis***

Data analysis was performed by Statistical Package for Social Sciences (SPSS) version 11.5 software (SPSS Inc., Chicago, IL, USA). Shapiro Wilk test was used to determine if continuous variables were distributed close to normal. Descriptive statistics were given as mean ± standart deviation or median (minimum-maximum) for continuous variables; whereas nominal variables were represented as number of cases or as percentage. Student's T test and Mann Whitney U test were used for the significance of differences between groups in terms of means and medians respectively. Nominal variables were analysed using Pearson's Ki Square test. Results were considered significant for p<0,05.

## Results

Baseline demographic, biochemical and echocardiographic parameters of the study population are provided in Table 1. FMF patients were older than healthy controls (p: 0.049). Median duration of

*Table 1. Baseline characteristics of the study population*

| | Control (n: 35) | FMF (n: 38) | p |
|---|---|---|---|
| **AGE** *[mean±sd]* | 28.1±9.1 | 32.8±11.0 | 0.049 |
| **SEX** *Female/Male* | 16/19 | 20/19 | 0.632 |
| **Hb** *[mg/dL]* | 14.9±1.87 | 14.7±1.80 | 0.652 |
| **WBC** *[×10³ ]* | 7.1 | 7.7 | 0.249 |
| **PLT** *[×10³ ]* | 258.8±84.88 | 284.4±62.34 | 0.142 |
| **Systolic blood pressure** *[mm Hg]* | 121.4±12.2 | 117.6±13.3 | 0.125 |
| **Diastolic blood pressure** *[mmHg]* | 80.1±5.4 | 79.5±6.0 | 0.119 |
| **HDL cholesterol** *[mg/dL]* | 48.4±11.2 | 47.3±12.8 | 0.550 |
| **LDL cholesterol** *[mg/dL]* | 110.43±25.5 | 105.7±28.4 | 0.346 |
| **Trigyceride** *[mg/dL]* | 132.6±19.9 | 129.9±23.3 | 0.130 |
| **Fasting glucose** | 78.7±10.4 | 80.1±12.3 | 0.244 |
| **Smoker** *[%]* | 34.5% | 37.4% | 0.080 |
| **Fibrinogen** *[mg/dL]* | 312.4 | 314.7 | 0.800 |
| **Fibrin dimer** *[µg/L]* | 160.8 | 118.5 | 0.306 |
| **ESR** *[mm/hr]* | 5.5 | 17.0 | <0.001 |
| **CRP** *[mg/dL]* | 3.0 | 2.6 | 0.912 |

*Hb: hemoglobin, WBC: white blood cell count, plt: platelet count, HDL: high density lipoprotein, LDL: low density lipoprotein, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein.*

the disease was 6 years (min: 1 yr – max: 35 yr) as well as the median duration of drug therapy (min: 1 yr – max: 35 yr). The ratio of women and men were similar between FMF and control groups (p: 0.062). None of the FMF patients had renal failure or biopsy proven amyloidosis although 10.3% of patients had non-nefrotic range proteinuria.

FMF patients and the control group were similar in terms of hemoglobin, white blood cell count, platelets or mean platelet volume (Table 1). Most of the inflammatory parameters like CRP, fibrinogen, fibrin dimer levels were not significantly different except erythrocyte sedimentation rate which was found to be higher in FMF patients ($p<0.001$).

Median CIMT in FMF group was higher than the control group (0.50 mm[IQR: 0.24] vs 0.45mm [IQR: 0.10] p: 0.016); however, this thickening was not found to be correlated with erythrocyte sedimentation rate, CRP or fibrinogen levels (B: 0.072 p: 0.587; B: 0.117 p: 0.371; B: 0.048 p: 0.706 respectively).

## Discussion

The role of inflammatory activity in the promotion and progression of atherosclerosis has been highlighted in many studies in the last decade (8). Inflammatory markers; hs-CRP and fibrinogen were identified as "emerging risk factors" for coronary artery disease by National Cholesterol education program Adult treatment Panel III Guidelines (9). Rheumatologic diseases most of which have an autoinflammatory basis have been investigated for the incidence and mechanisms of cardiovascular morbidity and mortality (10). CIMT which is a surrogate marker of atherosclerosis was found to be associated with future cardiovascular and cerebrovascular events (11). Patients with rheumatoid arthritis, systemic lupus erythematosus and ankylosing spondylitis had subclinical atherosclerosis represented by increased CIMT (12-14).

In this study we have demonstrated that CIMT was increased in uncomplicated FMF patients without any cardiovascular risk factors compared to the healthy controls despite Colchicine use. There are some other reports from different regions of our country with different results. Peru et al. have reported increased CIMT in children with FMF [15]; Ugurlu et al.(16) and Akdogan et al. (17) have demonstrated increased CIMT in adult FMF patients but Sari et al. (18) have reported no differences between FMF patients and controls with regards to CIMT.

The duration of the disease, presence of other risk factors of atherosclerosis, type and homogeneity of the genetic mutations as well as environmental factors may have caused these different results from the same country. To eliminate other cardiovascular risk factors, we have set strict disclusion criteria. The study population had no complications of FMF, like amyloidosis which causes end stage renal disease with established vascular alterations. All FMF patients in our study were on Colchicine treatment with different durations, however antiinflammatory activity of colchicine doesn't seem to prevent initiation of subclinical atherosclerotic vascular alterations.

CRP and fibrinogen levels were not significantly elevated in FMF group although eryhtrocyte sedimentation rates were higher; and we could not detect any association between inflammatory markers and carotid intima medial thickening. This may be partly due to the relatively small number of patients but still some other undetected mechanisms might play role in the atherosclerotic process in FMF.

Impact of subclinical atherosclerosis on future cardiovascular ischemic diseases in FMF patients remains unanswered. Langevitz et al. have reported that the frequency of ischemic heart disease in colchicine-treated FMF patients was comparable to that of the normal population but was significantly lower than in untreated patients with other inflammatory conditions (6). Still, larger, long-term, prospective studies are needed to demonstrate whether and to what extent subclinical atherosclerosis progresses to established coronary artery disease and if patients with increased CIMT should more aggressively be treated to prevent atherosclerotic coronary artery disease.

## References


1. *Livneh A, Langevitz P, Zemer D et al. Criteria for the diagnosis of familial Mediterranean Fever. Arthritis Rheum 1997; 40: 1879-1885*

2. *Touitou I. The spectrum of Familial Mediterranean Fever mutations. European J of Human Genetics 2001; 9: 473-83*

3. *Lachmann HJ, Sengül B, Yavuzşen TU, et al, Clinical and subclinical inflammation in patients with familial Mediterranean fever and in heterozygous carriers of MEFV mutations. Rheumatology 2006; 45: 746-750*

4. *Poredos P, Orehek M, Tratnik E. Smoking is associated with dose-related increase of intima-media thickness and endothelial dysfunction. Angiology 1999; 50: 201-208*

5. *Lorenz MW, Markus HS, Bots ML et al. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. Circulation 2007; 115: 459-67*

6. *Langevitz P, Livneh A, Neumann L et al. Prevalence of Ischemic Heart Disease in Patients with Familial Mediterranean Fever. Isr Med Assoc J 2000; 3: 9-12.*

7. *Altekin E, Demir I, Başarici et al. The relationship between carotid intima-media thickness and the presence and extent of angiographic coronary artery disease. Turk Kardiyol Dern Ars 2007; 35: 90-96*

8. *Libby P, Ridker PM. Inflammation and atherosclerosis: from population biology and bench research to clinical practice. J Am Coll Cardiol 2006; 48: A33-46*

9. *Pearson TA, Mensah GA, Alexander RW et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003; 107: 499-511*

10. *Tyrrell PN, Beyene J, Feldman BM et al. Rheumatic disease and carotid intima-media thickness: a systematic review and meta-analysis. Arterioscler Thromb Vasc Biol 2010; 30: 1014-26.*

11. *O'Leary DH, Polak JF, Kronmal RA et al. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular health study colloborative research group. N Eng J Med 1999; 340: 14-22*

12. *Gonzalez-Juanatey C, Llorca J, Martin J et al. Carotid intima-media thickness predicts the development of cardiovascular events in patients with rheumatoid arthritis. Semin Arthritis Rheum 2009; 38: 366-71*

13. *Schanberg LE, Sandborg C, Barnhart HX. Premature atherosclerosis in pediatric systemic lupus erythematosus: risk factors for increased carotid intima-media thickness in the atherosclerosis prevention in pediatric lupus erythematosus cohort. Arthritis Rheum 2009; 60: 1496-507.*

14. *Gonzalez-Juanatey C, Vazquez-Rodriguez TR, Miranda-Filloy JA et al. The high prevalence of subclinical atherosclerosis in patients with ankylosing spondylitis without clinically evident cardiovascular disease. Medicine (Baltimore)2009; 88: 358-65.*

15. *Peru H, Altun B, Dogan M et al. The evaluation of carotid intima-media thickness in children with Familial mediterranean fever. Clin Rheumatol 2008; 27: 689-694.*

16. *Ugurlu S, Seyahi E, Cetinkaya F et al. Intima-media thickening in patients with familial mediterranean fever. Rheumatology 2009; 48: 911-15.*

17. *Akdogan A, Calguneri M, Yavuz B et al. Are familial Mediterranean fever (FMF) patients at increased risk for atherosclerosis? Impaired endothelial function and increased intima media thickness are found in FMF. J Am Coll Cardiol 2006; 48: 2351-53.*

18. *Sari I, Karaoglu O, Can G et al. Early ultrasonographic markers of atherosclerosis in patients with familial mediterranean fever. Clin Rheumatol 2007; 26: 1467-73*



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# The rate of Pneumococcal Pneumonia in children aged 2-6, over the period of November 2011 – November 2012

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**Abstract**

This is a prospective-retrospective study to isolate the microbiological agent Streptococcus pneumoniae from nose and throat swabs of children patients at the Department of Pulmonology of the Pediatric Clinic KCU Sarajevo over a period of one year, specifically between November 2011 and November 2012. The microbiological analysis of nose and throat swabs was conducted at the Microbiology Laboratory KC Sarajevo. The samples were treated by the method of primary and secondary sample processing, and, where there was resistance to multiple antibiotics, then an automatic sample testing was done.

In our analysis, the sought microbiological agent – Streptococcus pneumoniae, was isolated in 37 out of the tested 577 patients (6.4%).

A total of 18 children patients had a severe form of pneumonia, while 1 child had the complication of pleuropneumonia with a deteriorated respiratory insufficiency requiring mechanical ventilation.

Our study has shown that streptococcus pneumonia may cause a serious threatening condition – respiratory insufficiency, which ultimately requires mechanical ventilation. That is why streptococcus pneumoniae immunization would provide protection from the invasive pneumococcal disease in children, with complications.

**Key words:** Streptococcus pneumoniae, Pneumococcal Pneumonia, respiratory insufficiency, mechanical ventilation.

## Introduction

Pneumococcal disease in children is the leading cause of pneumonia. Streptococcus pneumoniae may cause serious life-threatening conditions, such as pneumonia, pleuropneumonia, insuffitientio respiratoria, meningitis, sepsis. These types of infection are called invasive pneumococcal diseases. (1, 2).

Epidemiologically, the disease usually occurs in the winter period and in early spring, and is spread quickly by saliva drops.

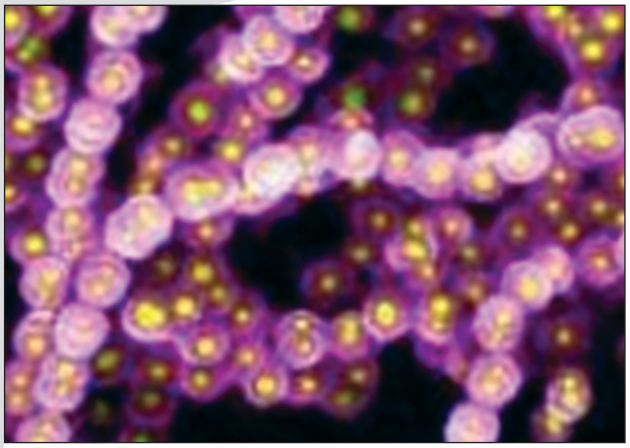

*Figure 1. Streptococcus pneumoniae*

Streptococcus pneumoniae (pneumococcus) is a Gram-positive bacteria, of an oval or round form, most often coming in pairs or short chains (3). It is coated with a capsule. It belongs to the group of α-hemolytic streptococci. The pneumococcus capsule is made of polysaccharides and it provides protection from the defensive system cell phagocytosis. S. pneumoniae is most commonly found in microscopic preparations in capsule-coated pairs, less

commonly in chains. It requires a string of amino acids and vitamins for reproduction, which is why it can be cultivated only on rich media such as blood and serum agar. On blood agar it creates tiny, smooth, shiny, round colonies with alpha-hemolysis. A significant biochemical property of most strains is inulin fermentation. There are around 80 types of pneumococci known so far (4).

Pneumococci inhabit the mucus membrane of the upper respiratory tract, where they belong to the normal bacterial flora. Due to other infections, e.g. influenza or defense system weakness, there may be cases of their excessive reproduction and spreading, which results in infections. It is spread by saliva drops. Pneumonia is developed in 4 stages.

*Stage I – the congestion stage* – lasts for several hours. Intra-alveolar capillaries are expanded and the alveoli start to fill with serous exudates, rare polymorphonuclears and an abundance of pneumococci.

*Stage II – the red hepatization stage* – the alveoli are filled with red blood cells, fibrin, serum and rare neutrophils.

*Stage III – the grey hepatization stage* – the alveoli are filled with a large number of neutrophils and fibrins. Capillaries in the alveoli are obstructed, thus providing for the formation of a D-L shunt /extracardial/, while blood flows in greater quantities through the preserved part of parenchyma, enabling a better hemoglobin saturation with oxygen. *Stage IV – the resolution stage* – The exudate is cheesy, the number of macrophages is on the increase, neutrophils are subjected to fat degeneration and necrosis, fibrin strips are lysated and vanish gradually. Polymorphonuclears phagocyte the pneumococci, while macrophages remove the debris from the alveoli.

Characteristic of pneumococcal pneumonia is a dark shade limited to one lobe (lobar pneumonia). Red blood cell sedimentation is accelerated, leukocytosis with predomination of neutrophil granulocytes and left skewing, while the acute inflammation reactants are positive. An examination of arterial blood gases may find hypoxemia (poor oxygen diffusion) and respiratory alkalosis (caused by hyperventilation). If there is no risk of resistance, the medicines of choice are penicillin, cephalosporins and macrolides, while vancomycin is a substitute. In case of the resistance risk (children, imunnosuppressed, patients on long-term antibiotic therapy), the medicine of choice is vancomycin, with cephalosporins acting as a substitute. As of recently, an increasingly important place in therapy is taken by antipneumococcal cinolons, mostly because a single medicine encompasses Gram-positive, Gram-negative and atypical pathogens, because the posology is simple and because larger concentrations are found in infection sites than in the serum.

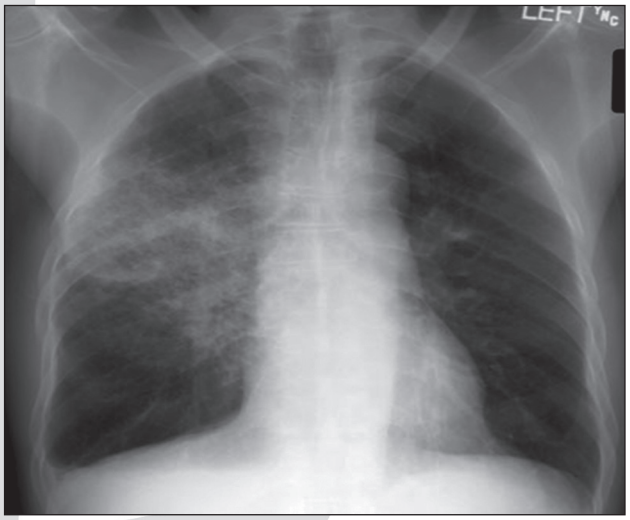

*Figure 2.*

***Study objective***

To monitor the microbiological agent Streptococcus pneumoniae over a period of one year, as taken from the nose and throat swab in children treated at the Department of Pulmonology, the Pediatric Clinic.

**Method**

Used as the relevant material may be nose and throat swab, sputum, liquor, blood (5). All children patients were admitted, hospitalized at the Department of Pulmonology, the Pediatric Clinic. For the purpose of our microbiological analysis, we used nose and throat swabs. The microbiological swab analysis was conducted at the microbiological laboratory of the KC Sarajevo. Samples were determined by the method of primary and secondary sample processing, and if there was resistance to multiple antibiotics, also conducted was automatic sample analysis. We conducted our microbiological throat swab examination in several stages: We took a sample from throat swab, and then

inoculation was done on a blood agar, incubation at the temperature of 37 °C, with a 24h period of waiting, which is when we got the results. This is the so-called primary sample processing method. If the culture was sterile after 24 hours, we moved on to the secondary sample processing method. After isolating the microbiological cause, the next phase is its identification, and determining its sensibility according to antibiogram.

Besides determining the presence of bacteria, also carried out was an automatic identification of the cause of disease within 24 h, using a pneumococci identification system (Vitek – Biomerix). This method was applied only in cases of sterile samples or in cases of sample resistance to antibiotics. In the automatic identification process, it took us 24 h for identification, sensibilization of the cause according to the antibiogram, and a minimal concentration sensitive to the medicine.

Applied here was a descriptive statistical analysis, percentage, through relevant tests 1. z hypothesis test.

## Results

Out of the 37 positive streptococcus pneumoniae findings, 17 children were carriers, 18 had developed clinical picture of pneumonia, while only 2 patients had the clinical picture of severe pneumonia, pleuropneumonia, with the outcome being disease progression to the stage of mechanical ventilation.

*Table 1. Display of 17 carriers: bronchitis 13, tonsillitis 3, 1 rhinitis*

| Carrier | Total | Percentage |
|---|---|---|
| Bronchitis | 13 | 4.81 % |
| Rhinitis | 1 | 0.37% |
| Tonsillitis | 3 | 1.11% |

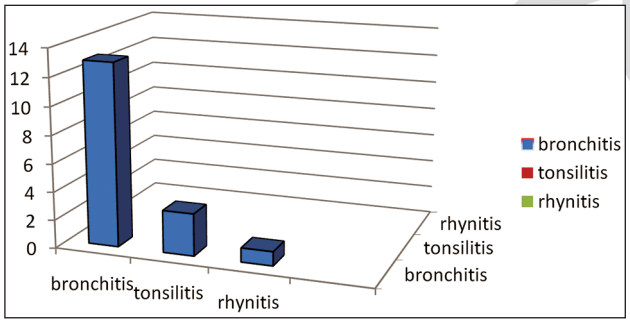


*Figure 3. Carrier: bronchitis 13, tonsillitis 3, 1 rhinitis*

## Results

In the prospective-retrospective analysis of microbiological samples of nose and throat swabs of the children patients admitted to the Department of Pulmonology of the Pediatric Clinic KCU Sarajevo, the sought microbiological cause of disease: Streptococcus pnaeumoniae, was isolated in 37 out of the admitted 577 patients (6.4%) during the period of 9 Nov. 2012 – 9 Nov. 2011.

Most of the patients had a positive microbiological bacteria streptococcus pnaeumoniae in nose swab in the following months: August 2012 (13.6 %), May 2012 (13.3%), April 2012 (13.3 %) and December 2011 (10.3%). Streptococcus pneumoniae was isolated from throat swab in one child only, and from ear swab in another. Of the total number of children (37), 23 of them were aged 2-5 (62.16%).

| | throat swabs | nasal swab | ear swab |
|---|---|---|---|
| nov.11 | 0 | 0 | 0 |
| dec.11 | 0 | 6 | 0 |
| jan.12 | 0 | 0 | 0 |
| feb.12 | 0 | 0 | 0 |
| mar.12 | 0 | 2 | 0 |
| apr.12 | 0 | 8 | 0 |
| maj.12 | 0 | 7 | 1 |
| jun.12 | 0 | 2 | 0 |
| jul.12 | 0 | 1 | 0 |
| avg.12 | 0 | 3 | 0 |
| sept.12 | 0 | 3 | 0 |
| oct.12 | 1 | 3 | 0 |

*Figure 4. Prospective- retrospective analysis of microbiological samples of nose and throat swabs taken from children patients admitted to the Department of Pulmonology, Pediatric Clinic Sarajevo*

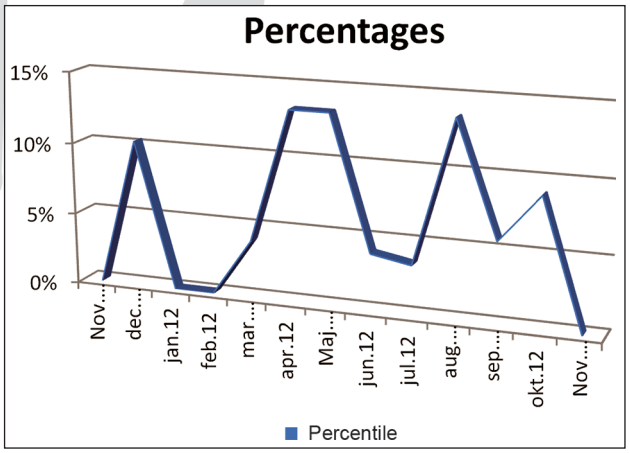


*Figure 5. Percentage in relation to microbiological finding (streptococcus pnaeumoniae isolation)*

*Table 2.*

| Month and year | Isolating Bacteriae pneumoniae from the sample | Number of children at the Department of Pulmonology per month and year | Percentage | Age | Town |
|---|---|---|---|---|---|
| November 2012 (starting from 1 Nov. – 9 Nov. 2012) | Throat swab: - (0)<br>Nose swab: -(0)<br>Ear swab: - (0) | 15 | 0 % | 0 | 0 |
| October 2012 | Throat swab: + (1)<br>Nose swab + (3)<br>Ear swab: - (0) | 33 | 9.0% | 2 years of age – 2 pac<br>4 - 2 pac | 3 children from Sarajevo<br>1 child from Kakanj |
| September 2012 | Throat swab: - (0)<br>Nose swab + (3)<br>Ear swab : - (0) | 52 | 5.7% | 2 - 1 child<br>1 – 2 children | Sarajevo |
| August 2012 | Throat swab: - (0)<br>Nose swab + (3)<br>Ear swab : (0) | 20 | 13.6% | 1 – 2 children<br>2 - 1 child | 2 children from Sarajevo<br>1 child from Olovo |
| July 2012 | Throat swab: - (0)<br>Nose swab + (1)<br>Ear swab: - (0) | 28 | 3.5 % | 2 – 1 child | Sarajevo |
| June 2012 | Throat swab: - (0)<br>Nose swab + (2)<br>Ear swab: - (0) | 50 | 4 % | 2 -1 child<br>6 – 1 child | Sarajevo |
| May 2012 | Throat swab: - (0)<br>Nose swab + (7)<br>Ear swab : + (1) | 60 | 13.3 % | 2 - 5 children<br>4 – 2 children<br>5 -1 child | 7 children from Sarajevo<br>1 child from Olovo |
| April 2012 | Throat swab: - (0)<br>Nose swab + (8)<br>Ear swab: - (0) | 60 | 13.3 % | 1 – 3 children<br>2 – 3 children<br>6 - 2 children | Sarajevo |
| March 2012 | Throat swab: - (0)<br>Nose swab: + (2)<br>Ear swab : - (0) | 50 | 4 % | 1 – 1 child<br>4 -1 child | Sarajevo |
| February 2012 | Throat swab: - (0)<br>Nose swab: -(0)<br>Ear swab: - (0) | 49 | 0 % | 0 | 0 |
| January 2012 | Throat swab: - (0)<br>Nose swab: -(0)<br>Ear swab: - (0) | 49 | 0 % | 0 | 0 |
| December 2011 | Throat swab: - (0)<br>Nose swab + ( 6)<br>Ear swab: - (0) | 58 | 10.3 % | 1 – 2 children<br>2 – 3 children<br>11 – 1 child | Sarajevo |
| November - until 9 Nov. 2011. | Throat swab: - (0)<br>Nose swab - ( 0)<br>Ear swab: - (0) | 53 | 0 % | 0 | 0 |

*Table 3. Children aged 2-5*

| Age | Number | Percentage |
|---|---|---|
| Children aged 2-5 | 23 | 62.16% |
| Total number of children | 37 | 100% |

*z-score=4,301163*

**Discussion**

The most representative sample of children is up to 5 years of age (6). A large percentage of this age is as with other authors.Streptococcus pneumoniae (pneumococcus) is a leading couse

of serious illness among children world wide (7). Before universal infant imunization with pneumococcal conjugate vaccine in the United States, streptococcus pneumoniae caused approximetely 17,000 cases of invasive disease each year among children younger than five years of age, including 700 cases of meningitis and 200 deaths (8).Our study also shows leading cause of pneumonia with streptococcus pneumoniae up to five years of age. z-score=4,301163

Of the total number of children (37), 23 of them were aged 2-5 (62.16%).

Pneumococcal disease is contracted by contact with a diseased person or with a carrier. In treating pneumococcal pneumonia and other infections, practice varies from one country to another, and depends on the form of illness, but usually administered is antibiotic therapy. Penicillin antibiotics are medicines of choice for invasive pneumococcal infections, but resistance to those medicines has been on the increase over the past years.

Since pneumococcal conjugate vaccine was added to the routine childhood immunization schedule in the United States, the incidence of invasive pneumococcal disease has declined by 60 to 90 percent in children younger than two years. Pneumococcal disease prevention by vaccination becomes ever more important (9).

## Conclusion

In our prospective-retrospective analysis of microbiological causes of disease from nose and throat swabs of children patients admitted to the Department of Pulmonology of the Pediatric Clinic KCU Sarajevo, the sought microbiological agent: Streptococcus pnaeumoniae was isolated in 37 out of the tested 577 patients (6.4%) within one year. A total of 18 children patients had a severe form of pneumonia, while 1 child had the complication of pleuropneumonia with a deteriorated respiratory insufficiency requiring mechanical ventilation. Since streptococcus pneumoniae annually kills almost 1 million children below 5 years of age, as such it may cause serious life-threatening conditions, which our study showed too. According to WHO, out of all health care measures, vaccination could have the greatest impact on reducing pneumococcal deaths. Pneumococcal vaccines provide protection from infection by various pneumococcal forms, also called serotypes. There exist more than 90 various serotypes of streptococcus pneumoniae discovered to date. Globally 10-15 serotypes are responsible for 80-90% cases of invasive pneumococcal diseases in children, which is why our recommendation too is immunization by vaccines containing antigens for 7 serotypes of streptococcus pneumoniae that are important for the global epidemiology of pneumococcal diseases. Also registered is a new vaccine containing antigens for 10 serotypes, and another providing protection from 13 serotypes. Based on all the aforementioned, immunization against streptococcus pneumoniae could provide protection from children's invasive pneumococcal diseases in 80-90% cases.

## References


1. *CDC. Preventing pneumococcal disease among infants and young children. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 2000 Oct 6; 49(RR-9): 1–38.*

2. *Tunkel AR, Hartman BJ, Kaplan SL, Kaufman BA, Roos KL, Scheld WM et al. Practice guidelines for the management of bacterial meningitis. Clin Infect Dis. 2004 Nov1; 39(9): 1267–8*

3. *Johnson HL, Deloria-Knoll M, Levine OS, Stoszek SK, Hance LF, Reithinger R.et al. Systematic evaluation of serotypes causing invasive pneumococcal disease among children under five: the pneumococcal global serotype project. PLoS Med 2010; 7.*

4. *Phongsamart W, Srifeungfung S, Dejsirilert S, et al. Serotype distribution and antimicrobial susceptibility of S. pneumoniae causing invasive disease in Thai children younger than 5 years old, 2000-2005. Vaccine 2007; 25: 1275.*

5. *Dagan R, Givon-Lavi N, Greenberg D, et al. Nasopharyngeal carriage of Streptococcus pneumoniae shortly before vaccination with a pneumococcal conjugate vaccine causes serotype-specific hyporesponsiveness in early infancy. J Infect Dis 2010; 201: 1570.*

6. *Kaplan SL, Barson WJ, Lin PL, et al. Serotype 19A Is the most common serotype causing invasive pneumococcal infections in children. Pediatrics 2010; 125: 429.*

7. *Nuorti JP, Whitney CG, Centers for Disease Control and Prevention (CDC). Prevention of pneumococcal disease among infants and children - use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine - recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2010; 59: 1.*

8. *Advisory Committee on Immunization Practices. Preventing pneumococcal disease among infants and young children. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2000; 49: 1.*

9. *National Center for Immunization and Respiratory Diseases. General recommendations on immunization --- recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2011; 60: 1.*

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# The waist-to-height ratio as a predictor of percent body fat in African American college students

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**Abstract**

The accumulation of body fat is highly correlated with metabolic syndrome, which contributes to an increased risk of cardiac diseases and type II diabetes. The purpose of this study was to determine which of the 4 indices [body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR)] was the best predictor of percent body fat (%BF) in African American college students. The subjects comprised 116 college students who visited a participating exercise physiology laboratory at the North Carolina A&T State University, Greensboro, United States, between June 1 and December 31, 2011. The BMI, WC, WHR, and WHtR were calculated or measured, and the %BF was determined using air-displacement plethysmography. For all subjects, the %BF showed a significant positive correlation with BMI ($r = 0.627$, $p < 0.001$), WC ($r = 0.657$, $p < 0.001$), WHR ($r = 0.199$, $p = 0.033$), and WHtR ($r = 0.728$, $p < 0.001$). Furthermore, the relative strength of these associations between %BF and the BMI (standardized coefficients; SC = 0.150), WC (SC = 0.342), WHR (SC = 0.285), and WHtR (SC = 0.692) remained significant. The SC values revealed that of the 4 variables, the WHtR and the BMI had the strongest and weakest associations with %BF, respectively. We conclude that WHtR is a better anthropometric index for %BF than BMI, WC, and WHR in African American college students.

**Key words:** Body mass index, waist circumference, waist-to-hip ratio, waist-to-height ratio, African American.

## Introduction

Obesity is becoming a serious public health problem in the United States. In 2011, according to a report from the Centers for Disease Control and Prevention (CDC), ~33.8% of adults and ~17% of children and adolescents aged 2–19 years in the United States of America were obese. Moreover, the incidence of obesity continues to increase (1).

Obesity is a major risk factor for cardiovascular diseases, diabetes, musculoskeletal disorders, and some cancers, and leads to a high mortality rate and disability (2-4). In addition, obesity aggravates chronic diseases, such as hypertension, arthritis, cholelithiasis, and hyper-cholesterol. Therefore, diverse efforts are required to prevent and control obesity (5).

The accurate determination of body fat compartments for the definition of obesity requires several techniques, including magnetic resonance imaging (MRI), dual energy x-ray absorptiometry, underwater weighing, dilution techniques, bioelectrical impedance analysis, air-displacement plethysmography (BOD POD), and computed tomography (CT) (6-7). However, while these techniques show reliability and accuracy in the measurement of body fat, they are relatively expensive and complex to use, inconvenient for the participant, impractical for routine clinical settings or large-scale studies, and not feasible for use in the field because they require large, specialized equipment (6-7).

In clinical settings, simple anthropometric measurements such as body mass index (BMI), waist circumference (WC), and waist-to-hip ratio (WHR) can be conveniently used to assess body composition, and these variables correlate reasonably well with laboratory-based measures of body composition (8-10).

Recently, several studies have reported that the waist-to-height ratio (WHtR) is a better predictor of cardiovascular risk factors, metabolic risk, abdominal fat distribution, and urinary albumin excretion rate than BMI, WC, and WHR (11-14). Even though the mechanisms that enable the WHtR to predict the wellness risk remain to

be authenticated, the relationship of this parameter with elevations in obesity is often suggested to underlie this predictive ability (15).

Previous studies have investigated Asian or Caucasian subjects (11-16), but no study has assessed the determination of these factors (e.g., metabolic risk) in African Americans. Thus, the purpose of this study was to determine which of the 4 indices (BMI, WC, WHR, and WHtR) was the best predictor of percent body fat (%BF) in African American college students.

## Methods

### *Subjects*

The subjects comprised 116 volunteer African American college students (60 males and 56 females) aged 17–39 years of age from North Carolina Agricultural and Technical State University, Greensboro, North Carolina, United States. The subjects visited an exercise physiology laboratory at the university between June 1 and December 31, 2011. All of the subjects signed a consent form to participate in this study, and the study procedures were approved by the Institutional Review Board at North Carolina A&T State University. The characteristics of the subjects are shown in Table 1.

*Table 1. The characteristics of the subjects (n= 116)*

| Variables | Male (n = 60) | Female (n = 56) |
|---|---|---|
| Age (years) | 21.57 ± 2.31 | 20.25 ± 3.29 |
| Height (cm) | 176.47 ± 6.51 | 165.18 ± 9.14 |
| Weight (kg) | 81.47 ± 14.92 | 67.98 ± 14.15 |
| BMI (kg/m$^2$) | 26.18 ± 4.55 | 24.81 ± 4.12 |
| WC (cm) | 83.16 ± 9.76 | 80.84 ± 10.38 |
| WHR (%) | 0.86 ± 0.05 | 0.82 ± 0.07 |
| WHtR (%) | 0.47 ± 0.06 | 0.49 ± 0.06 |
| Body fat (%) | 19.01 ± 7.90 | 24.16 ± 8.93 |

*Values are the mean ± standard deviation. BMI: body mass index; WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio*

### *Experimental procedures*

The BMI of each subject was calculated based on the height and weight. The WC of each subject was measured as follows: As the subject stood with the feet ~25–30 cm apart, a Gulick tension-tape measure was used to determine the WC at the portion of the trunk located halfway between the lower costal margin, or the bottom of the lower rib, and the iliac crest, or the top of the pelvic bone. The tape was fit carefully as the measurer stood beside the subject. By the use of standardized tension, care was taken not to compress any of the underlying soft tissues, with the circumference measured at the end of a normal expiration to the nearest 0.5 cm (17). The WHR was calculated based on waist circumference and measurements at the widest point of the hips. The WHtR was calculated using waist and height measurements.

For measuring %BF, the chamber pressure amplitudes of the BOD POD instrument (Life Measurement Inc., California, USA) were calibrated prior to each test using a 50-L calibration cylinder. Each subject wore a tight-fitting swimsuit or body suit, with the %BF determined in the chamber. A separate step was used to measure the thoracic gas volume. For acquiring this measurement, the subject sat quietly in the chamber, breathing through a disposal tube and filter, which were connected to the reference chamber at the back of the BOD POD apparatus. Following 4 or 5 breaths, the airway was blocked midway through the exhalation. The subject was then told to blow 3 rapid, light, panting breaths into the tube.

### *Statistical analysis*

The partial correlation coefficient was used to adjust for age when analyzing the correlation between %BF and the 4 examined indices (BMI, WC, WHR, and WHtR). Further, to assess the relative strength of these associations, we used multivariate regression analysis after adjusting for age. The statistical significance was set at $\alpha < 0.05$; SPSS ver. 20.0 (Chicago, IL, United States) was used for the analysis. The results are shown as the mean and standard deviation.

## Results

The results of the partial correlation analysis between %BF and the 4 indices (BMI, WC, WHR, and WHtR), as adjusted for age, are shown in Table 2. For all subjects, the %BF showed a significant positive correlation with BMI ($r = 0.627$, $p < 0.001$), WC ($r = 0.657$, $p < 0.001$), WHR ($r = 0.199$, $p = 0.033$) and WHtR ($r = 0.728$, $p < 0.001$).

*Table 2. The partial correlation coefficients of BMI, WC, WHR, and WHtR with percent body fat*

| Variables | Body fat (%) in African American college students (n = 116) | |
|---|---|---|
| | R | p-value |
| BMI (kg/m$^2$) | 0.627 | <0.001*** |
| WC (cm) | 0.657 | <0.001*** |
| WHR (%) | 0.199 | 0.033* |
| WHtR (%) | 0.728 | <0.001*** |

*BMI: body mass index; WC: waist circumference; WHR: Waist-to-hip ratio; WHtR: waist-to-height ratio*
**p < 0.05, ***p < 0.001, tested by partial correlation analysis after adjusting for age*

*Table 3. Comparison of the relative strengths of BMI, WC, WHR, and WHtR in predicting percent body fat*

| Variables | Body fat (%) in African American college students (n = 116) | | | |
|---|---|---|---|---|
| | Standardized coefficients (beta) | $R^2$ | F | *p*-value |
| BMI (kg/m$^2$) | 0.150 | 0.576 | 29.872 | <0.001*** |
| WC (cm) | 0.342 | | | |
| WHR (%) | 0.285 | | | |
| WHtR (%) | 0.692 | | | |

*BMI; Body mass index, WC; Waist circumference, WHR; Waist to hip ratio, WHtR; Waist to height ratio*
****p < 0.001, tested by multivariate logistic regression analysis after adjusting age*

A multivariate regression analysis of BMI, WC, WHR, and WHtR, as adjusted for age, was conducted to access the strength of the association between %BF and these 4 indices (Table 3). The association between BMI (standardized coefficient; SC = 0.150), WC (SC = 0.342), WHR (SC = 0.285), and WHtR (SC = 0.692) and %BF remained significant. Furthermore, the standardized coefficient values revealed that of the four variables, the WHtR and BMI showed the strongest and weakest associations with %BF, respectively.

## Discussion

Body fat accumulation is associated with cardiovascular disease and plays an important role in insulin resistance. Several previous studies have reported that WHtR is a better predictor than WC, WHR, and BMI for the evaluation of coronary disease and metabolic risk factors (11-13, 18). Furthermore, WHtR has been shown to be a better predictor for the evaluation of abdominal fat and obesity (14-15).

Based on previous studies with Caucasian and Asian subjects, we hypothesized that WHtR might also have a stronger association with the %BF than WC, WHR, and BMI in African Americans. Although BMI, WC, and WHtR showed similar correlations with %BF in the partial correlation analysis (Table 2), WHtR was revealed by multivariate regression analyses to be the best anthropometric index among these particular parameters in African American students (Table 3).

BMI has been generally used to define overweight and obesity (19). However, BMI has critical limitations because it does not reveal the condition of the body composition, such as fat-free mass, fat mass, and %BF (20-21). This study also supported previous studies showing that BMI has a weaker association with %BF compared to WC and WHtR (14).

Ferland *et al.* (1989) reported that the WHR was a good predictor of abdominal adipose tissue using CT (22). Further, Chan *et al.* (2003) reported that WC was a better predictor of abdominal adipose tissue than WHR using MRI (8). Recently, however, Wu *et al.* (2009) reported that WHtR was the best predictor of abdominal fat distribution when compared to BMI, WC, and WHR (14). Our study has also shown that WHtR is the best predictor of body composition not only in Caucasian and Asian individuals (8, 12, 14) but also in African Americans. This study has provided evidence that WHtR is an important surrogate marker of %BF in African Americans.

This study has some limitations. First, the sample of this study did not represent all African Americans, since all the participants resided in Greensboro, North Carolina, and were of college

age. Second, the number of participates in this study (n = 116) was not large. For this reason, further well-designed studies will need to be conducted to evaluate the extent to which these anthropometric variables represent the %BF among African Americans. However, unlike other studies, the subjects were African Americans, who represent a minority race and this is thus an advantage of this study. Additionally, according to the central limit theorem, a study likely has a normal distribution and is reliable if the number of subjects in each group is over 30 (23). Hence, because all of the experimental groups in this study had over 30 subjects, our results can be considered reliable.

## Conclusion

We conclude that WHtR is a better anthropometric index for %BF than BMI, WC, and WHR in African American college students.

## References


1. *Centers for Disease Control and Prevention. U.S. Obesity Trends. Centers for Disease Control and Prevention. Centers for Disease Control and Prevention. 2011. http://www.cdc.gov/obesity/data/trends.HTML*
2. *World Health Organization. Obesity and Overweight. Global Strategy on Diet, Physical Activity and Health. World Health Organization. 2011. http://www.who.int/mediacentre/factsheets/fs311/en/*
3. *Meseguer-Santamaría ML, Jiménez JM, Vargas MV. Analysis of social factors in the prevalence of obesity among women with disabilities. HealthMED. 2010; 4(4): 775-781.*
4. *Margina D, Gradinaru D, Panaite C, Danut C, Vladica M, et al. The association of adipose tissue markers for redox imbalance and the cardio-vascular risk in obese patients. HealthMED. 2011; 5(1): 194-199.*
5. *Wadden TA, Stunkard AJ. Handbook of obesity treatment (3rd ed.). New York: Guilford Press. 2002.*
6. *Lee SY, Gallagher D. Assessment methods in human body composition. Curr Opin Clin Nutr Metab Care. 2008; 11(5): 566-572.*
7. *Mattsson S, Thomas BJ. Development of methods for body composition studies. Phys Med Biol. 2006; 51(13): R203-228.*
8. *Chan DC, Watts GF, Barrett PH, Burke V. Waist circumference, waist-to-hip ratio and body mass index as predictors of adipose tissue compartments in men. Quarterly journal of medicine. 2003; 96: 441-447.*
9. *Deurenberg P, Yap M. The assessment of obesity: methods for measuring body fat and global prevalence of obesity. Bailliere Clin. Endocrinol. Metab. 1999; 13: 1-11.*
10. *Jia WP, Lu JX, Xiang KS, Bao YQ, Lu HJ, Chen L. Prediction of abdominal visceral obesity from body mass index, waist circumference and waist-hip ratio in Chinese adults: receiver operating characteristic curves analysis. Biomed Environ Sci. 2003; 16: 206-211.*
11. *Ho SY, Lam TH, Janus ED. Hong Kong Cardiovascular Risk Factor Prevalence Study Steering Committee. Waist to stature ratio is more strongly associated with cardiovascular risk factors than other simple anthropometric indices. Ann Epidemiol. 2003; 13: 683-691.*
12. *Hsieh SD, Yoshinaga H, Muto T. Waist-to-height ratio, a simple and practical index for assessing central fat distribution and metabolic risk in Japanese men and women. Int J Obes Relat Metab Disord, 2003; 27: 610-616.*
13. *Tseng CH. Waist-to-height ratio is independently and better associated with urinary albumin excretion rate than waist circumference or waist-to-hip ratio in Chinese adult type 2 diabetic women but not men. Diabetes Care, 2005; 28: 2249-2251.*
14. *Wu HY, Xu SY, Chen LL, Zhang HF. Waist to height ratio as a predictor of abdominal fat distribution in men. Chin J Physiol. 2009; 52(6): 441-445.*
15. *Ashwell M, Cole TJ, Dixon AK. Ratio of waist circumference to height is strong predictor of intra-abdominal fat. Br Med J. 1996; 313(7056): 559-560.*
16. *Wi-Young S, Ju-Han P, Jin P. Waist-to-height ratio and body mass index are than waist circumference and waist-to-hip ratio in elderly Korean women. HealthMED. 2012; 6(7): 2339-2343.*
17. *World Health Organization. Report of a WHO Consultation on obesity: Preventing and managing the global epidemic. Geneva. 1999.*
18. *Wu HY, Chen LL, Zheng J, Liao YF, Zhou M. Simple anthropometric indices in relation to cardiovascular risk factors in Chinese Type 2 diabetic patients. Chinese J. Physiol. 02007; 50: 135-142, 2007.*
19. *World Health Organization. BMI classification. World Health Organization. 2011 http://apps.who.int/bmi/index.jsp?introPage=intro _3.html*

20. *Rush EC, Goedecke JH, Jennings C, Micklesfield L, Dugas L, et al. BMI, fat and muscle differences in urban women of five ethnicities from two countries. Int J Obes (Lond). 2007; 31(8): 1232-1239.*

21. *Sluyter JD, Schaaf D, Scragg RK, Plank LD. Body mass index and percent body fat in a New Zealand multi-ethnic adolescent population. Int J Pediatr Obes. 2010 Mar 17. [Epub ahead of print].*

22. *Ferland M, Després JP, Tremblay A, Pinault S, Nadeau A, et al. Assessment of adipose tissue distribution by computed axial tomography in obese women: association with body density and anthropometric measurements. Br J Nutr. 1989; 61(2): 139-148.*

23. *Johnson RA, Bhattacharyya GK. Statistics: Principles and Methods. John Wiley & Sons, Inc. USA. 2010.*

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# Evaluation of multidetector Computed Tomography efficiency in patients underwent emergency intestinal resection due to acute abdomen

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**Abstract**

**Purpose:** The aim of this study was to evaluate the role and additional diagnostic contribution of multidetector computed tomography (MDCT) findings in patients with acute abdominal pain caused by small and large bowel pathologies that required emergency intestinal resection.

**Methods:** A total of 42 patients who were admitted to our hospital's emergency room between July 2011 and July 2012 with a complaint of acute abdominal pain caused by non-traumatic small and large bowel pathologies and underwent emergency intestinal resection were included to our study. The definitive diagnosis and MDCT findings of patients were retrospectively reviewed.

**Results:** Of these patients, 23 (54.8 %) were male and 19 (45.2 %) were female. The mean age was 49±3,4 years, ranging from 31 to 64 years. The sensitivity of MDCT in diagnosing intestinal pathologies leading to acute abdominal pain and required emergency intestinal resection was 93.7%, 91.7%, and 85.7% in patient groups of intestinal perforation, acute mesenteric ischemia, and intestinal obstruction, respectively. A total concordance between the MDCT findings and definitive diagnosis was found in 38 of 42 cases; partial discordance was seen in 2 of 42 cases (4.8%) and total discordance was seen in 2 of 42 cases (4.8%). The overall sensitivity of MDCT in the diagnosis of intestinal pathologies required emergency intestinal resection was found to be 90.5%.

**Conclusion:** MDCT is a rapid, reliable, highly accurate, and cost-effective modality in the differential diagnosis of small and large bowel pathologies leading to acute abdominal pain and requiring emergency intestinal resection.

**Key words:** Acute abdomen, emergency intestinal resection, multidetector computed tomography.

## Introduction

The term of 'acute abdomen' is a clinical condition characterized by a serious abdominal pain which develops within hours. It is frequently difficult to evaluate this condition clinically, and the conventional radiological findings and the laboratory results are non-specific. For patients who were admitted to an emergency room with acute abdominal pain, multidetector computed tomography (MDCT) is a quick, common and cost-effective radiological method for making a certain diagnosis. The non-traumatic abdominal pain is a common symptom in 1/3 of patients admitted to an emergency room [1]. For these cases, the differential diagnosis may vary from benign causes that may ameliorate spontaneously to life-threatening severe disorders requiring emergency surgery. The complications due to delays in diagnosis of the acute abdomen are common in routine clinical practices [2]. The function of radiology is to provide fast and accurate diagnosis and to reach a differential diagnosis the diseases that give good response to medical treatment and the diseases that require a surgery.

Acute abdomen is a clinical manifestation with a wide spectrum that arises from gastrointestinal, hepatobiliary, urological, gynecological and vascular pathologies. Among these, the gastrointestinal causes play an important role and may need intestinal resection due to impairment of clinical status [3]. Although it is believed that the radiological evaluation of luminal organs is limited, the bowels and the mesenteric vessels can be examined in details with the contribution of multiplanar

and three-dimensional images obtained by MDCT and CT-angiographic investigations. A markedly faster imaging is achieved with MDCT technology, in which movement and respiratory artifacts can be eliminated; furthermore, it is also possible to examine the distal branches of intestinal vessels by rapid administration of contrast agent [4].

The aim of this study was to evaluate the role and additional diagnostic contribution of MDCT findings in patients with acute abdominal pain caused by small and large bowel pathologies which required emergency intestinal resection.

## Materials and Methods

In our study, a total of 42 patients who were admitted to our hospital's emergency room between July 2011 and July 2012 with a complaint of acute abdominal pain caused by non-traumatic small and large bowel pathologies and underwent emergency intestinal resection were retrospectively reviewed. The demographic data, medical history, physical examination, laboratory and radiological findings, surgery notes and pathological diagnosis of these patients were obtained. The patients who underwent intestinal resection due to penetrating or blunt trauma and had a primary surgical repair were excluded from the study. All patients underwent 4-slice MDCT (HiSpeed; GE Medical Systems, Milwaukee, Wisconsin, USA) using basal and venous scans (at 70–90 s from contrast agent injection); the arterial phase (at 30–40 s) was carried out only in the event of suspected ischemic or vascular disease. Scans were performed with the following parameters: KV=120, mAs=modulation with range 60–230 mAs. Iodine contrast agent was administered intravenously as follows: volume, 120–150 ml; rate, 2.5 ml/s; concentration, 350 mg I/ml. In all cases, the contrast agent injection was followed by the injection of 40 ml saline solution at 2–3 ml/s.

MDCT examination was evaluated by two expert radiologists using axial image and multiplanar reconstruction (MPR), coronal, sagittal, or oblique according to the localization of the disease; cases of disagreement were reviewed jointly, with the collaboration of a third experienced radiologist. The MDCT diagnosis was compared with definitive diagnosis obtained after surgery and pathological examination.

## Results

A total of 42 patients who applied to the emergency room of our hospital with a manifestation of acute abdomen, and underwent an emergency intestinal resection between July 2011 and July 2012 were included in our study. Of these patients, 23 (54.8%) were male and 19 (45.2%) were female. The mean age was 49±3,4 years, ranging from 31 to 64 years.

The most frequent cause of acute abdomen requiring emergency surgery were adhesions lead to intestinal obstruction and perforated duodenal ulcer in our patients. All patients underwent emergency surgery within 48 hours after admission. Eight patients (19%) had post−operative morbidity. The most frequent complication was surgical site infection, which occurred in 5 patients ( 11.9%), followed by pneumonia in 3 patients (7.1%). One patient (2.4%) died within 1 month after the operation due to sepsis related to pan-peritonitis.

The definitive diagnoses of patients, which were made based on surgical procedure and pathological examination, were divided into 3 main groups: a) intestinal obstruction (total of 16 patients); b) acute mesenteric ischemia (AMI) for 12 patients; c) intestinal perforation (total of 14 patients).

Intestinal obstruction was most often caused by adhesion (n = 7). Other etiologies of intestinal obstruction were colonic malignancy (n = 4), inguinal hernia (n=2), internal hernia (n = 1), intussusception (n=1), and small-bowel tumor (n = 1). Among the cases of intestinal perforation, 7 cases were small intestinal perforations and 7 cases were large intestinal perforations. The most common cause of intestinal perforation was perforation due to large-bowel tumor (n = 3), caecum perforation due to acute appendicitis (n = 2) followed by colon diverticulitis (n = 2). Duodenal perforations were found in 7 cases of perforated duodenal ulcer.

The MDCT findings of patients with intestinal obstruction included increased transverse diameter of proximal bowel segments of obstruction, normal diameter of distal bowel loops of stenosis with a transition zone between distal collapsed bowel loops and proximal dilated bowel loops (Figure 1). The fluid distension in the proximal bowel loops of obstruction functions as a natural contrast agent. In case of tumors are the reason of

intestinal obstruction, contrast enhancement is observed in the solid tumors after intravenous contrast agent administration (Figure 2).

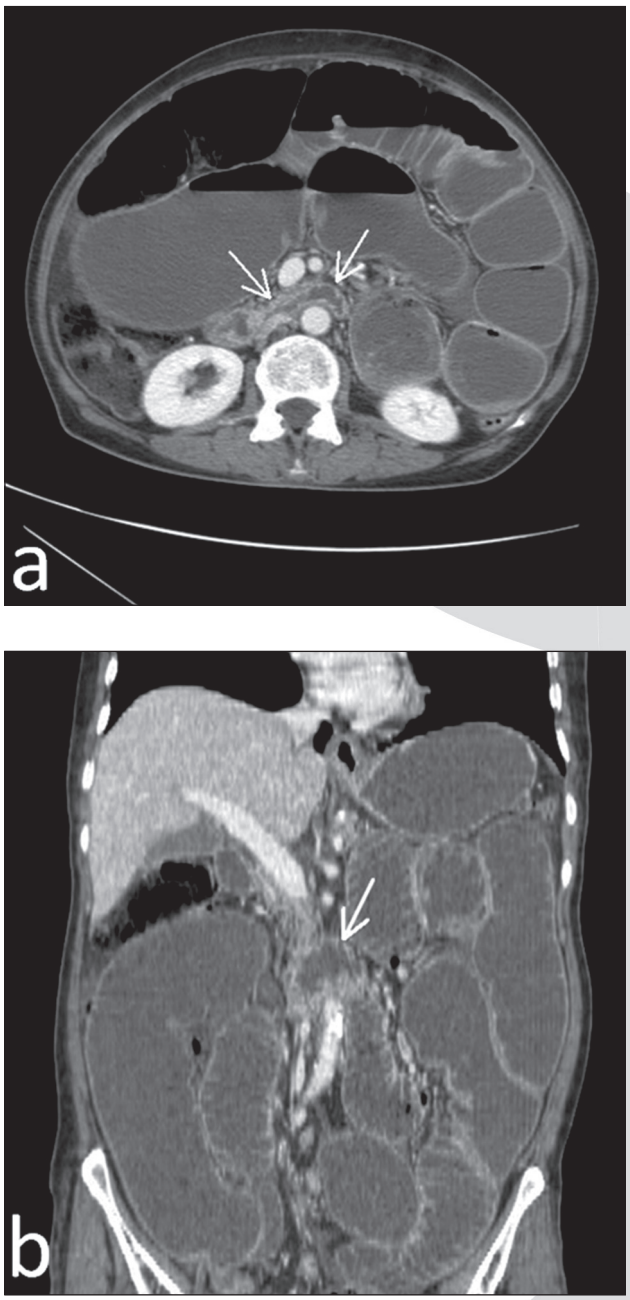


*Figure 1. a) Axial, b) Coronal reformatted image of a 47-year-old woman 1 year after abdominal surgery presents with a 1-day history of worsening abdominal pain, distention, nausea, and vomiting. MDCT scan with iv contrast agent of the abdomen demonstrates markedly dilated fluid filled loops of small bowel. Transition from markedly dilated loops of small bowel to collapsed loops of distal small bowel (arrows) is present with no identifiable etiology, making adhesions the likely diagnosis.*

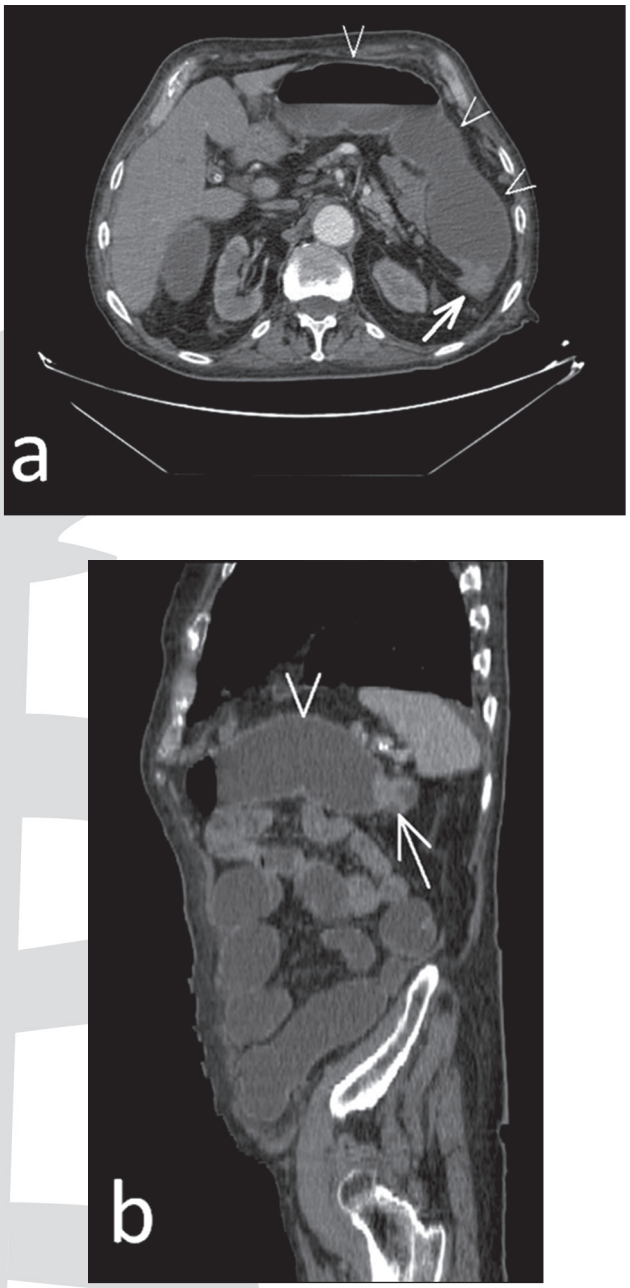


*Figure 2. a) Axial, b) Sagittal reformatted image of a 51 year-old male patient shows obstruction due to tumoral mass in the splenic flexure (arrow), a marked dilatation and air-fluid levels in transverse colon (arrowhead).*

Hernias were the third common cause of acute intestinal obstruction in our patients. In cases of strangulated hernia, compromise of the blood supply is present, resulting in thickening of bowel loops. Adjacent inflammatory changes can be seen at MDCT in association with small bowel obstruction (Figure 3). The MDCT diagnosis of foramen of Winslow hernia is established by the presence of bowel posterior to the stomach, which is charac-

teristically displaced to the left. In our case MDCT examination showed dilated intestinal loops in the lesser sac (Figure 4).

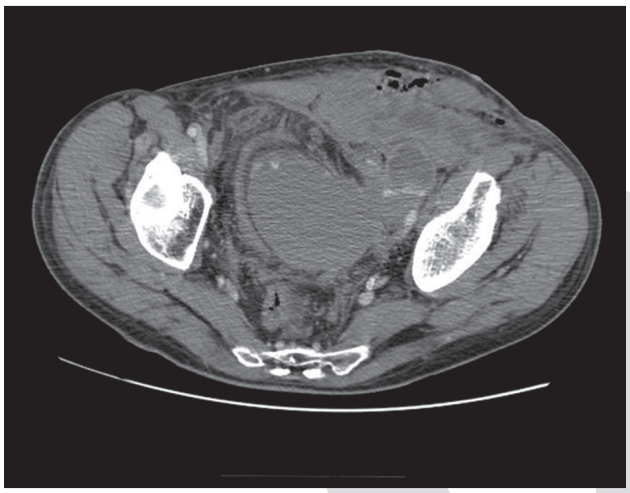

*Figure 3. Axial MDCT scan shows incarcerated left inguinal hernia leads to small bowel obstruction. CT scan obtained at a lower level demonstrates an incarcerated small bowel segment in an inguinal hernia.*

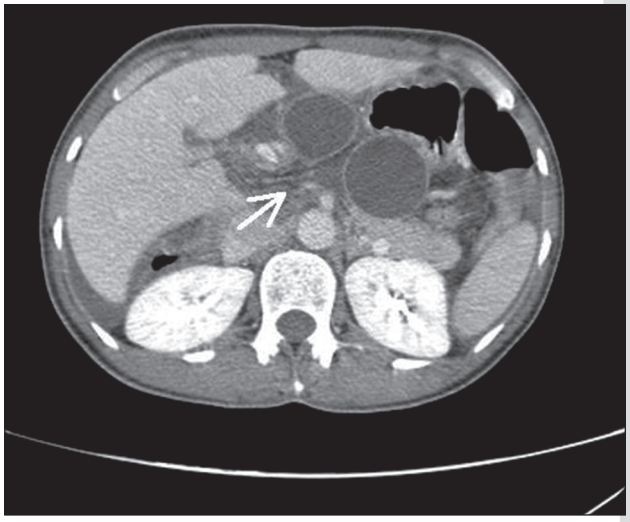

*Figure 4. Axial MDCT image shows dilated intestinal loops in the lesser sac indicated foramen of Winslow hernia (arrow). This finding was confirmed surgically.*

In our patients group with AMI, occluded or stenosed vascular structures were clearly seen in axial and multiplanar reformatted MDCT images in arterial phase after intravenous contrast agent administration (Figure 5). The presence of gas in bowel wall (pneumatosis intestinalis) and portal-venous system can be determined by MDCT images, which is one of the late findings of AMI and an indicator of poor prognosis (Figure 5a, 6).

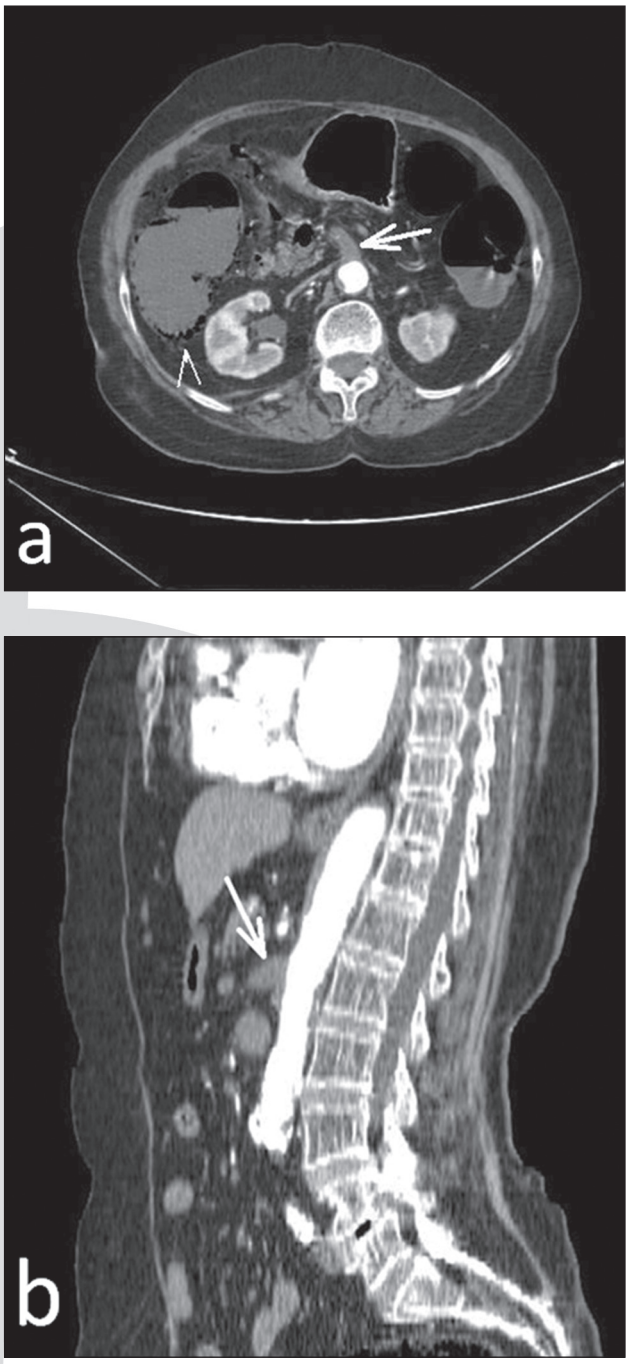


*Figure 5. a) Axial, b) Sagittal reformatted image of MDCT scan with iv contrast agent administered to 56-year-old female patient shows thrombus causing a total filling defect in the root of superior mesenteric artery (arrow), and the presence of pneumatosis intestinalis in colon wall in the hepatic flexure (arrowhead).*

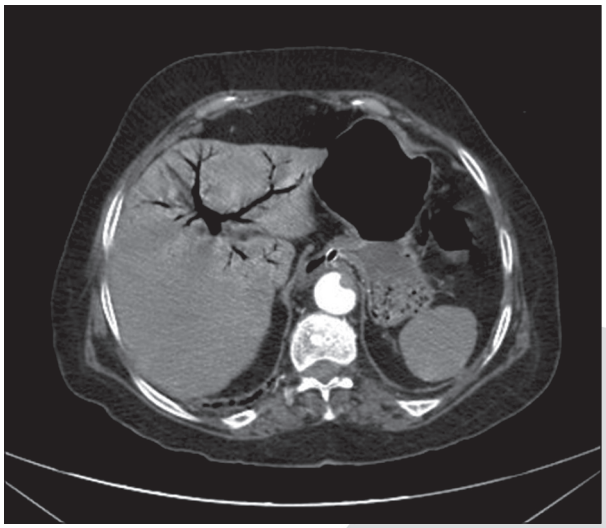

*Figure 6. Axial CT scan of liver with iv contrast agent administered to 63-year-old female patient shows the presence of gas in intra-hepatic portal venous system.*

The MDCT findings frequently observed in our patients with intestinal perforation were the presence of intra-abdominal extraluminal free air bubbles and the presence of intra-abdominal extraluminal free fluid (Figure 7).

In the present study, the sensitivity of MDCT in diagnosing small and large bowel pathologies leading to acute abdominal pain and required emergency intestinal resection was 93.7%, 91.7%, and 85.7% in patient groups of intestinal perforation, AMI and intestinal obstruction, respectively.

Total concordance between the MDCT diagnosis and definitive diagnosis was found in 38 of 42 cases in this study; partial discordance was seen in 2 of 42 cases (4.8%) and total discordance was seen in 2 of 42 cases (4.8 %) (Table 1). In the present study, the overall sensitivity of MDCT in the diagnosis of small and large bowel pathologies required emergency intestinal resection was found to be 90.5%.

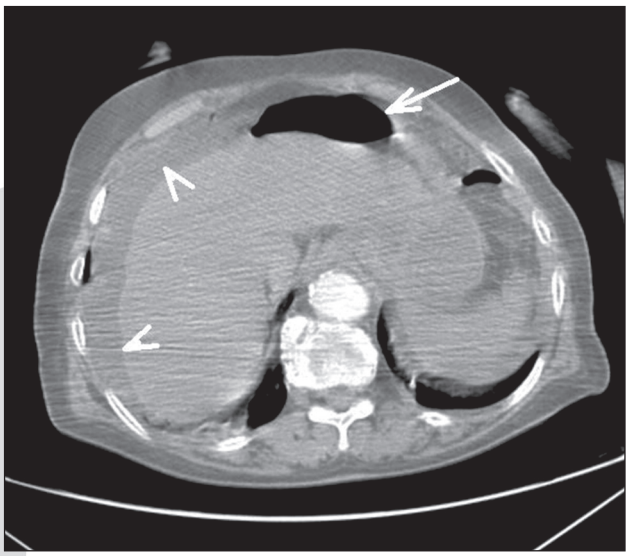

*Figure 7. Axial CT scan of 37-year-old female patient shows extraluminal free air adjacent to left lobe of liver (arrow), and free fluid in perihepatic region (arrowhead) are observed.*

## Discussion

Over the years, MDCT has gained a key role in the diagnosis of small and large bowel pathologies caused acute abdomen. It has reduced the use of conventional radiology due to its clear benefits of fast execution, broad view, and objective interpretation, as well as its ability to enable differential diagnosis in case of the initial clinical suspicion is not confirmed [5,6].

In a study evaluating the effect of MDCT on treatment of patients with acute abdominal pain, it

*Table 1. Comprasion of sensitivity of MDCT diagnosis and definitive diagnosis in patients with non-traumatic acute abdominal pain and required emergency intestinal resection.*

| Definitive diagnosis | Number of patients (n) | Number of concordance MDCT/Definitive diagnosis (n) | Number of discordance MDCT/Definitive diagnosis | | Sensitivity (%) |
|---|---|---|---|---|---|
| | | | Partial Discordance (n) | Total Discordance (n) | |
| Intestinal obstruction | 16 | 15 | 1 | 0 | 93.7 |
| Acute mesenteric ischemia | 12 | 11 | 0 | 1 | 91.7 |
| Intestinal perforation | 14 | 12 | 1 | 1 | 85.7 |
| Total | 42 | 38 | 2 | 2 | 90.5 |

*MDCT: Multidetector computed tomography*

has been demonstrated that MDCT altered the previously scheduled treatment in one third of cases, reduced the hospitalization time and directed to new alternative diagnosis in approximately 25% of those cases [7]. In the current study, MDCT leaded to new alternative diagnosis by excluding other possible diagnoses in 9 patients (21.4%) of our cases, which is similar to those reported previously.

Intestinal obstruction is a common cause of abdominal pain, accounting for 8% of all emergency room visits for abdominal pain and 20% of surgical admissions. MDCT often facilitates the identification of the cause of an intestinal obstruction, whether it is extrinsic or intrinsic to the bowel. The most common cause of intestinal obstruction in developed countries is adhesions, with the vast majority secondary to prior abdominal or pelvic surgery, which is comparable with recent studies from Turkey [8, 9]. In the previous studies from our country, the most common etiological causes were reported as strangulated hernias (32.2 - 54%), malignancies (10.2 - 27%), and adhesions (16 - 23%), respectively [11,12].

Changing of the etiological distribution of intestinal obstruction in our population may be resulted from several reasons including recent developments in the field of health, the increasing rates of the reaching health care services and also high sensitivity and specificity of MDCT in accurate diagnosing of intestinal obstruction.

In our study, the sensitivity of diagnosing intestinal obstruction with MDCT was 93.7%,which was comparable with the recent literature. In studies of intestinal obstruction, sensitivity and specificity of CT are reported as 71 to 95 and 68 to 98%, respectively [12,13]. This broad range can be explained by considering that MDCT is highly effective in recognizing complete intestinal obstruction (and their underlying cause) but it has considerable limits in identifying partial obstructions especially those caused by adhesions. Standard CT scans emerged as an important preoperative imaging modality for evaluation of intestinal obstruction almost two decades ago [14]. Many studies since then, have consistently demonstrated high sensitivities (90%-96%) and specificities (96% to 100%) of MDCT at determining the presence of intestinal obstruction [14,15].

AMI is an infrequent but important cause of acute abdominal pain, as it is associated with an average mortality rate of 71% [16]. Therefore, MDCT plays a critical role in the diagnosis of patients with AMI. The majority of AMI cases are due to thromboembolic disease affecting the superior mesenteric artery (SMA), with 50% secondary to embolus and 20% secondary to thrombosis. SMV occlusion is less common, responsible for 5% to 10% of cases [17].

Mesenteric occlusive ischemia due to thromboembolic disease is best evaluated with MDCT angiography technique [18]. An occlusive thrombus can be identified as a hypodense filling defect within the proximal portion of the SMA, usually adjacent to a site of preexisting calcified atherosclerotic plaque. An occlusive acute embolus also appears as a hypodense filling defect, but is typically located at arterial branching points. Three-dimensional reconstructions and volume-rendered images of the mesenteric vasculature can often be quite helpful for confident diagnosis. [17]

MDCT is useful in patients with AMI, resulting from whether intestinal occlusion or other diseases; its sensitivity and specificity vary from 67% to 100% and 83% to 100%, respectively, in the recent literature [13,18]. Taourel et al. evaluated the accuracy of MDCT in the diagnosis of AMI in a study population of 39 consecutive patients and found a sensitivity of 64% and a specificity of 92% using MDCT [19]. In another study, Kirkpatrick et al. reported a maximum sensitivity of 96% and a specificity of 94% in diagnosing mesenteric ischemia evaluated by MDCT [20]. In our study, MDCT correctly diagnosed AMI in 11 of 12 patients with a sensitivity of 91.7%. The results of the present study were similar to previous studies evaluating AMI by MDCT [19,20].

Although some recent studies reported that the sensitivity of MDCT for the diagnosis of AMI was found to be 100% [21], our results did not reach these values. This may be as a result of the difference between these studies in which either a 16-slice or 40-slice was used and the our study in which a 4-slice MDCT scanner was used.

The perforation of gastrointestinal tract is an emergency situation and patients with perforated bowel usually present with obvious signs of peritonitis, often as a complication of peptic ulcer disease or diverticulitis, or following endoscopic procedures [22]. The patients mostly require sur-

gical intervention and diagnosis is generally based on radiological imaging methods. Determination of the presence, the level and the cause of perforation is important for proper evaluation and surgical planning [6].

Several authors have shown that MDCT is the most valuable imaging technique for detecting free intraperitoneal air [23,24]. In a retrospective serie of 14 patients with perforated peptic ulcers reported by Chen et al. [25], the sensitivity of MDCT for showing free air was 100%, but the site of perforation could only be determined by MDCT in five patients (36%). Maniatis et al. [26] reported a sensitivity of 85.5% in a serie of 76 patients presenting with bowel perforation secondary to various causes. In our study, multiplanar reconstruction images were routinely performed, which we found to be useful for determining the exact location of bubbles of extraluminal air and defects in the bowel wall. The overall accuracy in predicting bowel perforation was 85.7% in our study. These results are similar to previously published retrospective series [26-28].

A recent study reveals that the most common causes of death in patients underwent emergency abdominal surgery were sepsis related to pan-peritonitis and pneumonia [29]; another previous study also reports that post-operative pneumonia, cardiac complications and sepsis accounted are the most frequent reasons of deaths in patients had abdominal surgery [9]. In our patients group, only one patient (2.4%) died within 1 month after the surgery due to sepsis related to pan-peritonitis which is one of the most common cause of death among these patients group in the literature.

In our study, MDCT correctly identified the diagnosis in 38 of 42 patients with a sensitivity of 90.5% when considering only those cases where there was complete agreement between MDCT diagnosis and the definitive diagnosis. Our results were comparable with previous studies reported in the literature for acute intestinal diseases considered. However, to the best of our knowledge, no data have been published evaluating the accuracy of MDCT in diagnosing acute intestinal pathologies that required emergency intestinal resection in the recent literature to compare with.

In conclusion, MDCT has gained widespread acceptance as a rapid, reliable, highly accurate, and cost-effective modality in the differential diagnosis of small and large bowel pathologies which lead to acute abdominal pain and require emergency intestinal resection. With recent advances in multi-planar and reformatted three-dimensional images, MDCT plays an increasingly important role in the evaluation of intestinal causes allowing a fast and precise differential diagnosis and therefore a timely treatment, which is mandatory to limit morbidity and mortality.

## References


1. *Urban BA, Fishman EK. Tailored helical CT evaluation of acute abdomen. Radiographics 2000; 20: 725-49.*
2. *Max P. Rosen, Daniel Z. Sands, H. Esterbrook Longmaid, III, Kevin F. Reynolds, Michelle Wagner, and Vassillios Raptopoulos. Impact of Abdominal CT on the Management of Patients Presenting to the Emergency Department with Acute Abdominal Pain. Am. J. Roentgenol 2000; 174: 1391-6.*
3. *Fishman EK. High-resolution three-dimensional imaging from subsecond helical CT data sets: applications in vascular imaging. AJR Am J Roentgenol 1997; 169: 441-3.*
4. *Zorger N, Schreyer AG. Multidetector computerized tomography in abdominal emergencies. Radiologe. 2009; 49: 523-32.*
5. *Grassi R, Di Mizio R, Pinto A, Romano L, Rotondo A. Serial plain abdominal film findings in the assessment of acute abdomen: spastic ileus, hypotonic ileus, mechanical ileus and paralytic ileus. Radiol Med 2004; 108: 56–70*
6. *Leschka S, Alkadhi H, Wildermuth S, Marincek B. Multidetector computed tomography of acute abdomen. Eur Radiol 2005; 15: 2435–47.*
7. *Taorel P, Baron MP, Pradel J, et al: Acute abdomen of unknown origin: Impact of CT on diagnosis and management. Gastrointest Radiol 1992; 17: 287-91.*
8. *Lvoff N, Breiman RS, Coakley FV, et al. Distinguishing features of self-limiting adult small bowel intussusception identified at CT. Radiology 2003; 227: 68-72.*
9. *Kapan M, Onder A, Polat S, Aliosmanoglu I, Arikanoglu Z, Taskesen F, et all. Mechanical Bowel Obstruction and Related Risk Factors on Morbidity and Mortality J Curr Surg 2012; 2: 55-61*

10. *Kaya B, Uctum Y, Kutanis R. Mechanical intestinal obstruction: etiology and clinical results. Turkish Journal of Surgery 2010; 26: 3-7.*

11. *Uludag M, Akgun I, Yetkin G, Kebudi A, Isgor A, Sener A. [Factors affecting morbidity and mortality in mechanical intestinal obstruction]. Ulus Travma Acil Cerrahi Derg. 2004; 10(3): 177-84.*

12. *Peck JJ, Milleson T, Phelan J. The role of computed tomography with contrast and small bowel follow-through in management of small bowel obstruction. Am J Surg 1999; 177: 375–8.*

13. *Silva AC, Pimenta M, Guimaraes L S. Small bowel obstruction: what to look for. Radiographics 2009; 29: 423-9.*

14. *Torreggiani WC, Harris AC, Lyburn ID, al-Nakshabandi NA, Zwirewich CV, Brenner C, et. al. Computed tomography of acute small bowel obstruction: pictorial essay. Can Assoc Radiol J 2003; 54: 93-9.*

15. *Brandt LJ, Boley SJ. AGA technical review on intestinal ischemia. American Gastrointestinal Association. Gastroenterology 2000; 118: 954-68.*

16. *Levy AD. Mesenteric ischemia. Radiol Clin North Am. 2007; 45: 593-9.*

17. *Wiesner W, Mortele KJ, Glickman JN, et al. Pneumatosis intestinalis and portomesenteric venous gas in intestinal ischemia: Correlation of CT findings with severity of ischemia and clinical outcome. AJR Am J Roentgenol 2001; 177: 1319-23.*

18. *Balthazar EJ, Liebeskind ME, Macari M. Intestinal ischemia in patients in whom small bowel obstruction is suspected: evaluation of accuracy, limitations, and clinical implications of CT in diagnosis. Radiology 1997; 205: 519–22.*

19. *Taourel PG, Deneuville M, Pradel JA, et al. Acute mesenteric ischemia: diagnosis with contrast-enhanced CT. Radiology 1996; 199: 632–6*

20. *Kirkpatrick ID, Kroeker MA, Greenberg HM. Biphasic CT with mesenteric CT angiography in the evaluation of acute mesenteric ischemia: initial experience. Radiology 2003; 229: 91–8.*

21. *Yikilmaz A, Karahan OI, Senol S, Tuna IS, Akyildiz HY. Value of multislice computed tomography in the diagnosis of acute mesenteric ischemia. European Journal of Radiology 2011; 80: 297–302.*

22. *Kuhlman JE, Fishman EK, Milligan FD, Siegelman SS: Complications of endoscopic sphincterotomy: Computed tomographic evaluation. Gastrointest Radiol 1989; 14: 127-32.*

23. *Stapakis JC, Thickman D: Diagnosis of pneumoperitoneum: Abdominal CT vs. upright chest film. J Comput Assist Tomogr 1992; 16: 713-6.*

24. *Earls JP, Dachman AH, Colon E, Garrett MG, Molloy M. Prevalence and duration of postoperative pneumoperitoneum: sensitivity of CT vs left lateral decubitus radiography. AJR 1993; 161: 781–5*

25. *Chen CH, Huang HS, Yang CC, Yeh YH. Features of perforated peptic ulcers in conventional computed tomography. Hepatogastroenterology 2001; 48: 1393–6.*

26. *Maniatis V, Chryssikopoulos H, Roussakis A, et al. Perforation of the alimentary tract: evaluation with computed tomography. Abdom Imaging 2000; 25: 373–9.*

27. *Kim HC, Shin HC, Park SJ, et al. Traumatic bowel perforation: analysis of CT findings according to the site and the elapsed time since accident. Clin Imaging 2004; 28: 334–9.*

28. *Catalano O. Computed tomography in the study of gastrointestinal perforation. Radiol Med (Torino) 1996; 91: 247–52.*

29. *Fukuda N, Wada J, Niki M, Sugiyama Y, Mushiake H. Factors predicting mortality in emergency abdominal surgery in the elderly. World J Emerg Surg 2012; 7: 12.*

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# Histological study on the hazardous effects of ethanol on liver and spleen in Swiss albino mice

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**Abstract**

Considering that ethanol caused number of health problems in the world , the present study was initiated to investigate the histological hazardous effects of ethanol on the liver and spleen. Animals were divided into 4 groups, the first group served as a control group, the second, third and fourth received 1,2 and 6 ml/kg/bw of 70% ethanol respectively. At the day 5 post treatment, the liver and spleen sections were prepared for the histological study. Ethanol intake induced marked histological alterations in the liver and spleen that correlated with the dose taken, low dose induced liver and spleen injury mainly as cytoplasmic degeneration in liver and abnormal structure of spleen, whereas, high doses of ethanol resulted in fibrosis in liver and splenomegaly.

**Key words:** Ethanol, mice, liver, spleen.

## Introduction

Ethanol has been a part of the human diet for centuries. However, its consumption in excess can result in a number of health problems, most notably liver damage , Ethanol cannot be excreted and must be metabolized, primarily by the liver (Berg *et al.,* 2002). *Alcoholic Liver Disease (ALD)* is a blanket term in which conditions related specifically to the liver and alcohol use fall under. The most prevalent types of Alcoholic Liver Disease, or ALD, are fatty liver, alcoholic hepatitis, and cirrhosis. Often, as people continue to drink heavily, they progress from fatty liver to hepatitis to cirrhosis. All three of the disorders can occur together ( Robert et al., 2004). *Fatty Liver*, which occurs after acute alcohol ingestion, is generally reversible with abstinence and is not thought to predispose to any chronic form of liver disease if abstinence and/or moderation are maintained (Kyrsten, 2004). It is estimated by the National Institutes of Health that about 20 percent of alcoholics and heavy drinkers develop fatty liver, or steatosis (Robert et al., 2004). Other sources estimate that 90-100 percent of patients with heavy drinking will develop this disease (Ismail and Riely, 2006). The condition can lead to death if alcohol consumption is not reduced or stopped, Alcohol can be a factor in several other forms of liver disease not specifically attributed to it, and may interact with risk factors for other forms of liver disease. An example of this is people with alcohol-related cirrhosis are at a higher risk of developing liver cancer. Those with Hepatitis B or C accompanied with heavy drinking are at a much higher risk of cirrhosis than with heavy drinking alone (Robert et al., 2004). Clinical evidence supports a correlation between excessive alcohol consumption and certain bacterial infections. For example, alcoholics who have developed cirrhosis of the liver are predisposed to spontaneous bacterial peritonitis. Phagocytes are an important defense against infection in this part of the body and defects in phagocytic cell function, observed in many alcoholics, may predispose these individual to peritoneal infection (Roselle, 1992). There is considerable evidence indicating that ethanol consumption alters immune system function and leads to increased susceptibility to infections and neoplastic diseases (Nath and Szabo, 2009; Lau et al., 2009; Nava-Aguilera et al., 2009; Szabo and Mandrekar, 2009).

## Materials and Methods

### *Animals and experimental design*

Male swiss mice weighed 25 to 30 g were obtained from King Saoud university animal house. Mice were provided with water and balanced diet *ad libitum.* 20 adult swiss mice were randomly divided into 4 groups of 5 mice in each as following:

- Group 1: control group ( normal mice without treatment).
- Group 2 : mice treated with 1ml /kg/bw of 70% ethanol orally by stomach gavage for consecutive 5 days.
- Group 3 : mice treated with 2ml/kg/bw of 70% ethanol orally by stomach gavage for consecutive 5 days.
- Group 4: mice treated with 6ml/kg/bw of 70% ethanol orally by stomach gavage for consecutive 5 days.

***Histological examination***

Mice were sacrificed in the sixth day of the beginning of the experiment, livers and spleens were collected and cut into small pieces and fixed 10% neutral buffered formalin, then embedded in paraffin and sectioned. The sections were stained with Hx&E stain for histopathological examination and photographing.

**Results**

***1. Liver***

The liver of normal mice (untreated) consists of a vast interanastomosing network of hepatocytes arranged in single-cell thick plates separated by vascular sinusoids. The hepatocytes along with vascular channels form organized micro structures which serve as structural and functional units. The liver is composed of innumerable lobules, each of which is a hexagonal structure consisting of a central vein surrounded by radiating hepatocyte plates. However, another concept of a functional unit defines an acinus as the functional unit in relation to terminal portal branches and terminal hepatic venule. Portal tracts surround the classical lobules. An interlobular portal vein is also shown (Figure 1).

Histopathological examination of liver of mice received 1ml/kg/bw of 70% ethanol orally for 5 days showed hazardous effects on liver represented by appearance of many necrotic foci (N), vacuolar degeneration (vc) in the cytoplasm of hepatocytes, some cells showed abundant nuclei, others showed pyknotic (P) nuclei where others appeared without nuclei. Dilatation in blood sinusoids with kuppfer (K)cells besides to existence of lymphocytic infiltration foci were abundant (L) (Figures 2a&2b). Sections stained by masson's trichrome technique showed wide dilated and branched central vien (CV) (Figure 2c),moreover, Figure 2d revealed congested and dilated central vein with destructed wall and surrounded by necrotic areas.

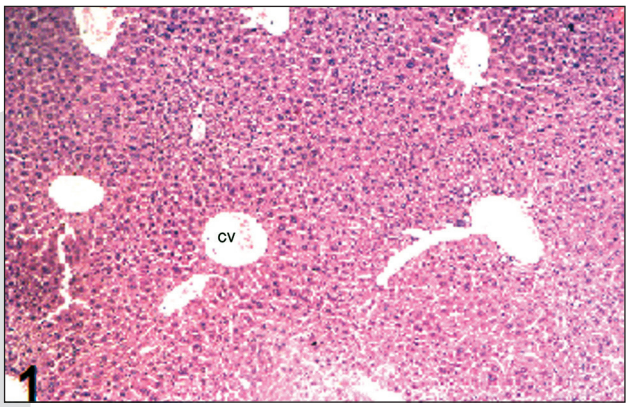


*Figure 1. Uuntreated liver section showed normal central vein (CV) surrounded by normal hepatocytes (mag.x200 H&E)*

Examination of liver sections of mice received oral administration of 2ml/kg/bw of 70% ethanol for 5 days showed more complicated alterations remarked by destruction in the vein wall surrounded by aggregations of lymphocytes and necrotic areas (N), in addition, pyknotic nuclei were abundant in some cells, and other cells showed complete degeneration in its nuclei, moreover, sections revealed fusion of cells due to degeneration of cell walls (Figures 3a&3b). Whereas, trichrome stained sections (Figures 3c&3d) displayed wide necrotic areas were filled with erythrocytic exudates, in addition to, wide dilated blood sinusoids with kupfer cells, dilated portal vein appeared surrounded by concentric layers of collagenous fibers with lymphocytes in between.

Livers of mice received oral administration of 6ml/kg/bw of 70% ethanol revealed the worst hazardous features in liver tissues compared with previous doses mainfested by cytoplasmic and nuclear degeneration in hepatocytes, some cells showed pyknotic nuclei (p), dilatation in blood sinusoids in addition to appearance of kupfer cells in the sinusoids, presence of necrotic areas besides to blood exudates among liver cells and appearance of blood congestion in dilated central vein (Figure 4a), moreover, Figure 4b showed some aggregations of lymphocytes in liver tissue. Concentrated

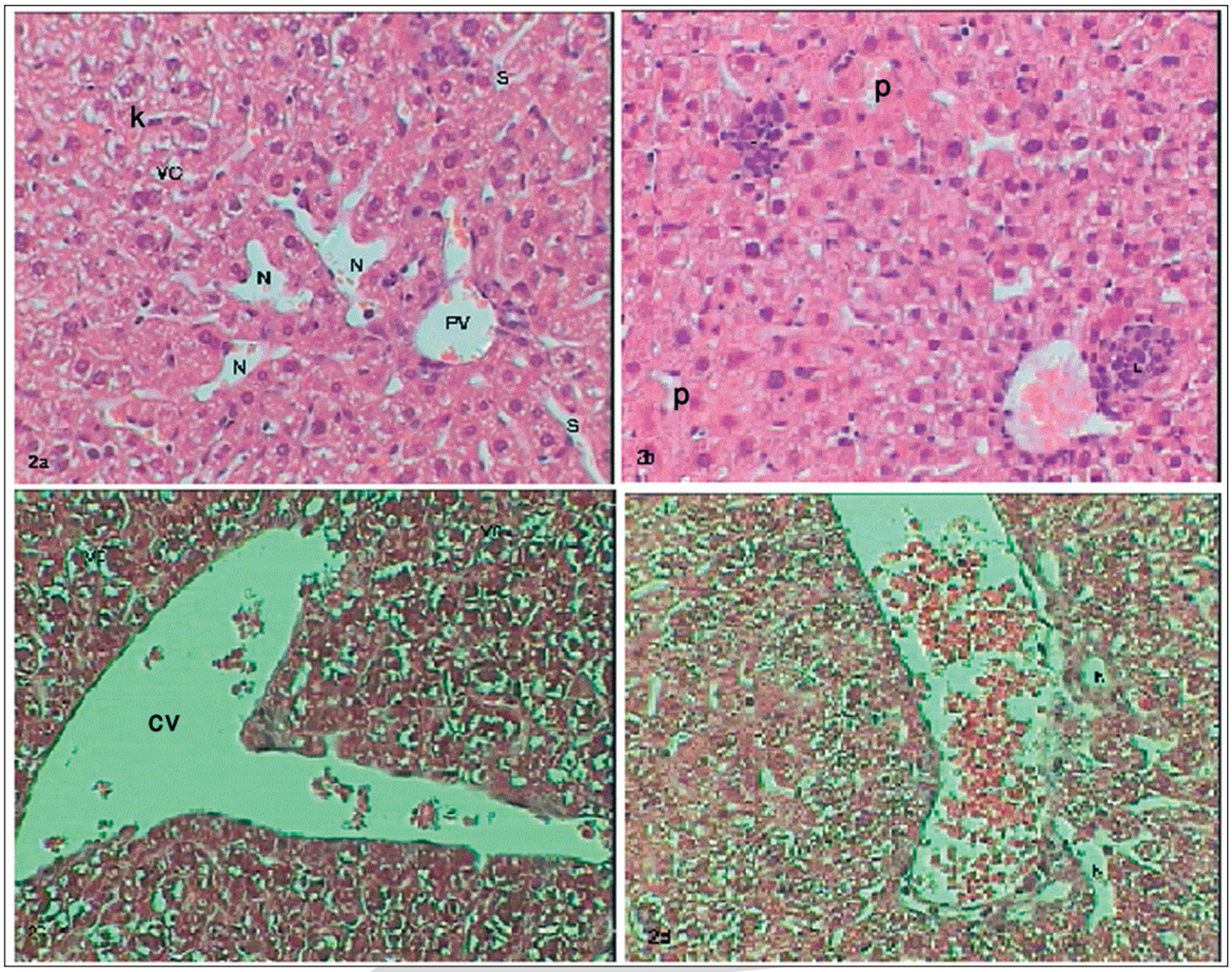


*Figure 2. Liver sections treated with 1ml/kg/bw of ethanol showed marked changes (2a mag.x400 H&E) necrotic areas (N), vacuolar degeneration (VC), kupffer cells (K)and blood sinusoids (S) (2b mag.x400 H&E) lymphocytic infiltration (L) and pyknotic nuclei (P). (2c mag.x400 M.Tr.) dilated central vein, (2d mag.x400 M.Tr.) dilated central vein with erythrocytic congestion surrounded by necrotic foci (N).*

layers of collagenous fibers were deposited in the tissue accompanied by lymphocytic infiltration and blood exudates, moreover, dilated central vein surrounded by thick concentric layers of collagenous fibers besides to presence of necrotic areas were seen (Figures 4c&4d).

### *2. Spleen*

Spleen is a large lymphoid organ that contains two main compartments known as "the white pulp and the red pulp". Spleen is covered with a fibrous capsule from which trabeculae enters into the parenchyma. The fibrous trabeculae ramify throughout the spleen and form supportive sheathing around blood vessels. The white pulp consists of lymphoid follicles, Each B-lymphoid follicle contains a distinct marginal zone of lymphocytes (outer rim of lymphocytes) around the mantle zone of the follicle (inner rim of lymphocytes). The marginal zone consists of loosely arranged lymphocytes. This 'perifollicular zone' is the boundary between white and red pulp and serves as the area where macrophages are abundant, The macrophages serve as the 'custom officers' for the newly entered red and white blood cells and particulate material. The red pulp is the area of spleen in between white pulp and consists of open sinuses and cellular cords, Splenic sinuses are open vascular spaces lined by a discontinuous layer of endothelial cells and supported by a fenestrated basal lamina and reticular fibers. The surrounding cellular splenic cords provide a tissue framework maintaining the network of sinuses.

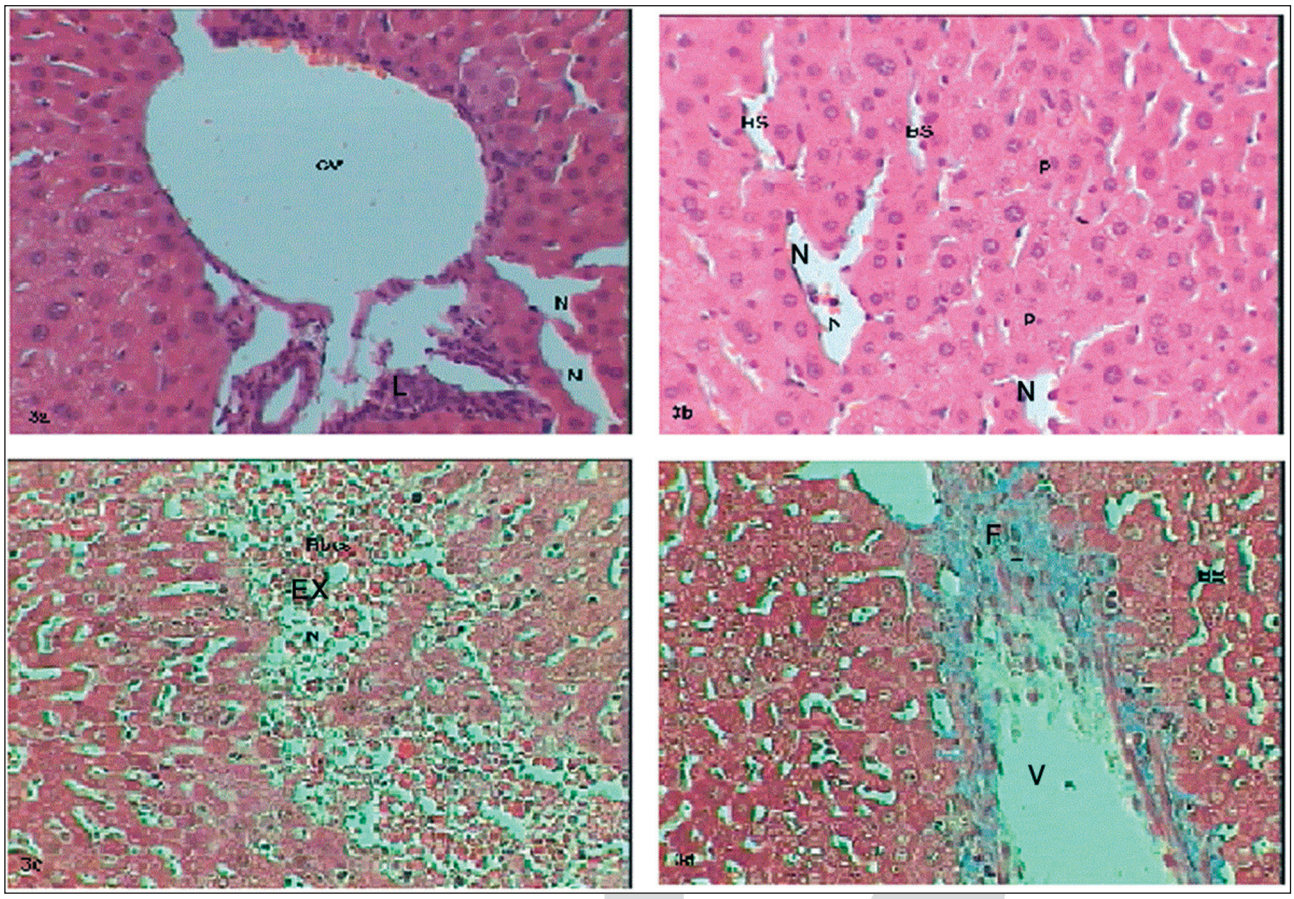


*Figure 3. Liver sections treated with 2ml/kg/bw of ethanol showed more complicated alterations (3a mag.x400 H&E) central vein (CV) with destructed wall surrounded by necrotic areas (N) and lymphocytic infiltration (L). (3b mag.x400 H&E) dilated blood sinusoids (bs), pyknotic nuclei of hepatocytes (P), necrotic foci (N). (3c mag.x400 M.Tr.) wide necrotic area(N) filled with erythrocytic exudates (Ex). (3d mag.x400 M.Tr.) dilated vein (V) surrounded by fibrotic layer (F).*

Examination of mice spleen sections received 1 ml/kg/bw oral administration of 70% ethanol for 5 days showed marked changes in the spleen tissue represented by expansion of red pulp due to venous congestion, presence of number of resident macrophages to get rid of abnormal red blood cells and cellular inclusions due to the venous congestion (Figures 6a &6b). Dilatation in venous sinuse that lined with endothelial cells besides to necrotic foci appeared in Figures 6c and 6d.

Spleen sections of mice received oral administration of 2 ml/kg/bw of 70% ethanol revealed abnormal architecture of spleen tissue remarked by distorted lymphoid follicles (white pulp) with resident macrophages in the marginal zone of the follicles, increasing the expansion of red pulp due to the increasing of venous congestion in addition to the dilatation in the venous sinuses ( Figures 7a, 7b & 7c), whereas, Figure 7d showed wide necrotic areas in the lymphoid follicle due to the degeneration of B and T lymphocytes.

By increasing the dose of 70% ethanol to 6ml/kg/bw spleen sections showed very hazardous effects on the spleen represented by splenomegaly due to severe congestion that venous sinuses completely disappeared because of filling of red blood cells, in other side, distorted white pulp appeared with small sized lymph follicles surrounded by necrotic areas (Figures 8a &8b).

## Discussion

Ethanol, which is a weak anesthetic, is toxic for both humans and animals, and exerts important negative effects upon the liver, brain, heart, skeletal muscle, pancreas, hematological and immune systems, gastrointestinal apparatus and endocrine system (Rottenberg, 1992, Rodés et al.,1990 and

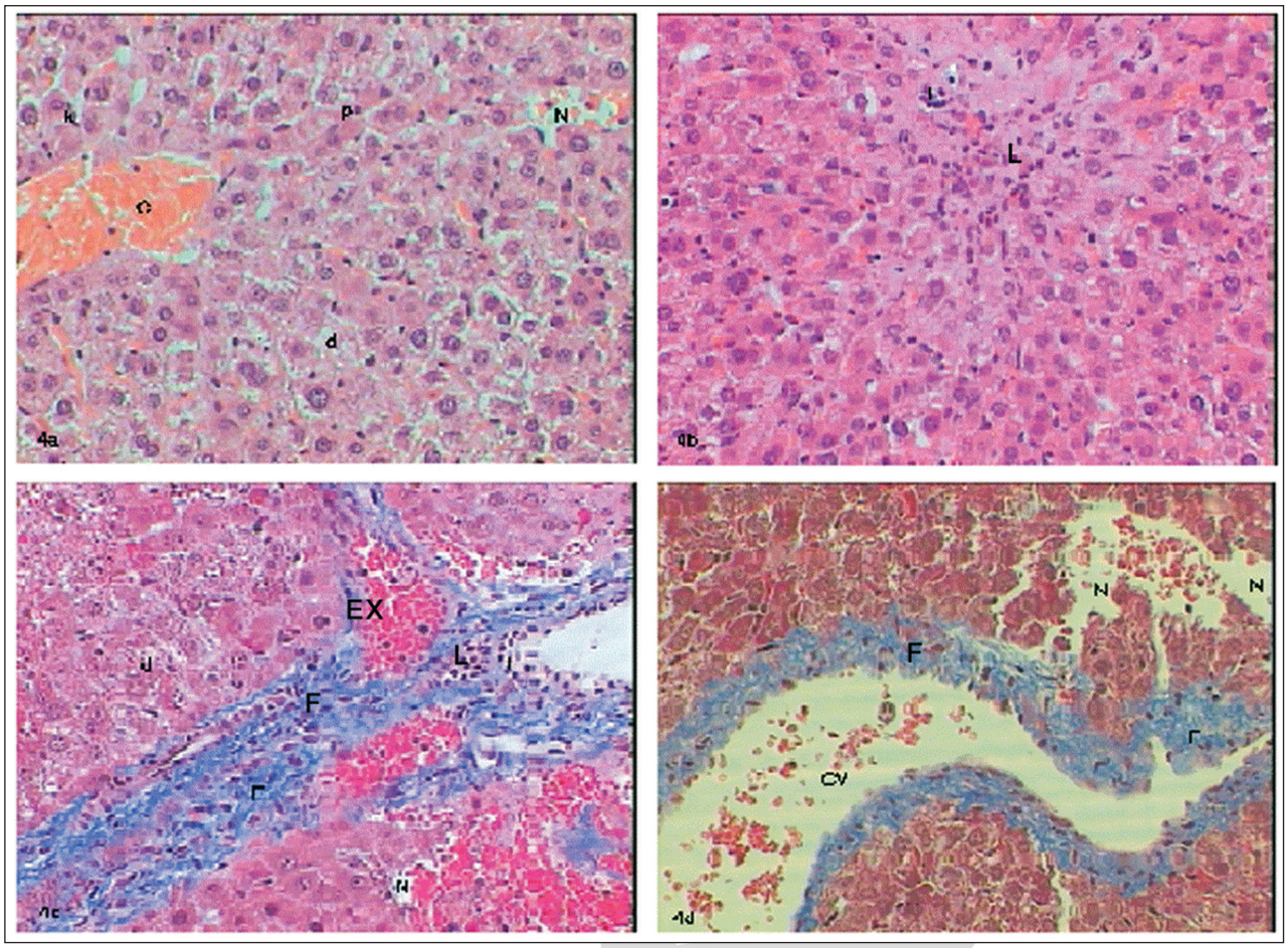


*Figure 4. Liver sections treated with 6ml/kg/bw of ethanol showed hazardous feathures (4a mag.x400 H&E) necrotic area (N) , pyknotic nuclei (P) , hepatocytic degeneration (d) (4b mag. X400 H&E) aggregations of lymphocytes (L) (4c mag.x400 M.Tr.) collagenous fibers (F) stained blue in the tissue, lymphocytic infiltration (L) and blood exudates (EX). (4d mag.x400 M.Tr.) dilated central vein (CV) surrounded by collagenous fibers (F) and wide necrotic areas (N).*

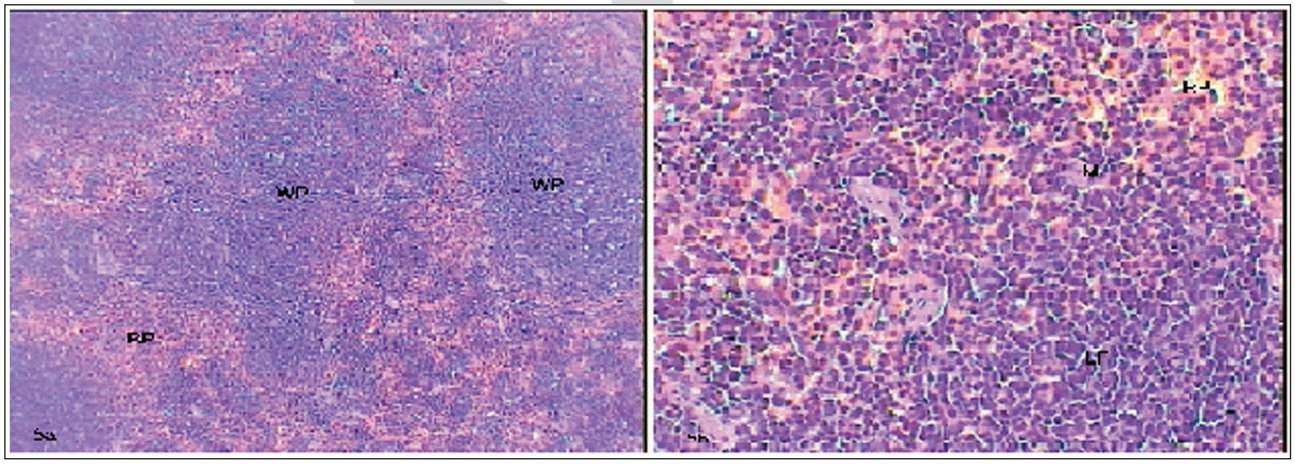


*Figure 5. Untreated spleen sections showed normal architecture (5a mag.x100 H&E) white pulp (WP) and red pulp (RP) (5b magx400 H&E)lymphoid follicle consists of lymphocytes (L), macrophages (M)*

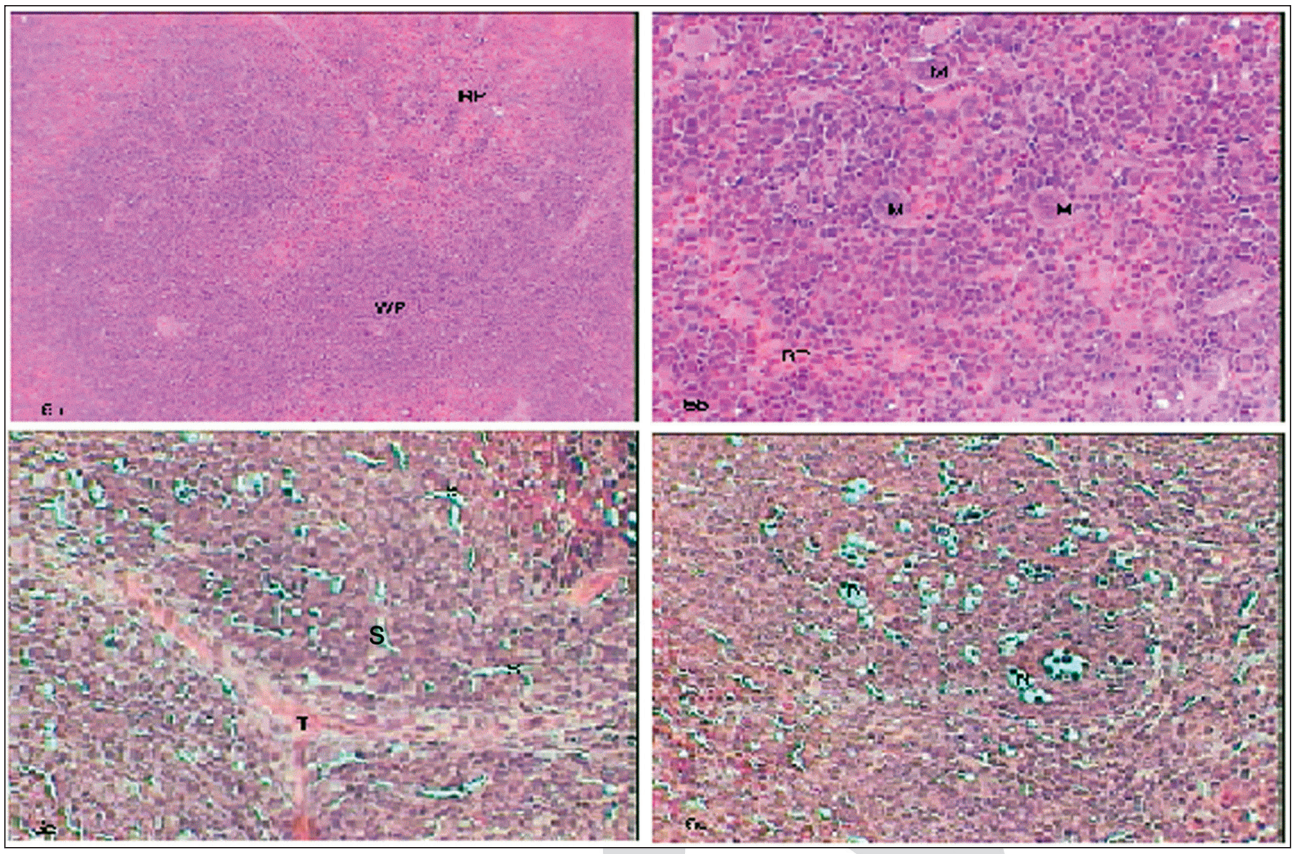


*Figure 6. Treated spleen sections with 1ml/kg/bw of ethanol showed marked changes (6a mag.x100 H &E) expansion of red pulp (RP), shrinkage of white pulp (WP). (6b mag.x400 H &E) number of macrophages (6c mag.x400 M.Tr.)trabeculae (T) , dilated venous sinusoids (S) (6d mag.x400 M.Tr.) necrotic foci (N).*

Bujanda et al.,1999). Alcohol can cause liver damage in the form of steatosis or fatty liver, fibrosis and liver cirrhosis. In general, the amount and duration of alcohol abuse correlate with the presence and severity of liver damage, at least as regards the initial stage of fatty liver (Becker et al., 1996). The present experimental study runs in full agreement with the previous studies that ethanol intake exerts hazardous effects upon liver and spleen, moreover, the amount of ethanol received correlates with severity damage of liver and spleen, it was observed that liver and spleen of mice received high doses of 2,6 ml/kg/bw of ethanol showed severe damage in the tissues compared to that received 1ml/kg/bw of ethanol.

Many studies proved that fatty changes, necrosis and inflammation were prominent in the livers of rats fed on ethanol( Gouillon et al., 2000). reported that Wistar rats livers exposed to 10% v/v ethanol for 12 weeks showed important changes in hepatic trabecular structure and increased hepatocytes with cytoplasmatic vacuoles (Brzóska *et al.,*2003 and Ito et al.,2007) . The characteristic Liver Alcoholic Disorder (steatosis and fibrosis is not observed when ethanol concentrations are low(Harrison & Burt, 1993). In accordance with the previous results, the present study proved that administration of low doses of ethanol affected mice liver histology represented by necrosis and cytoplasmic vacuoles in liver, but there is no fibrosis was detected in the liver tissue, whereas, sections treated with high doses of ethanol showed accumulation of fibers that increased by increasing the dose, the appearance of fibrosis might be due to increasing of hepatocytic degeneration which replaced by fibrosis. Moreover, low doses of ethanol caused harmful effects on the spleen manifested by expansion of red pulp and increasing number of macrophages to get rid of cellular inclusions as the dose increased, the expansion and congestion of red pulp increased whereas, the white pulp decreased and distorted which resulted in splenomegaly.

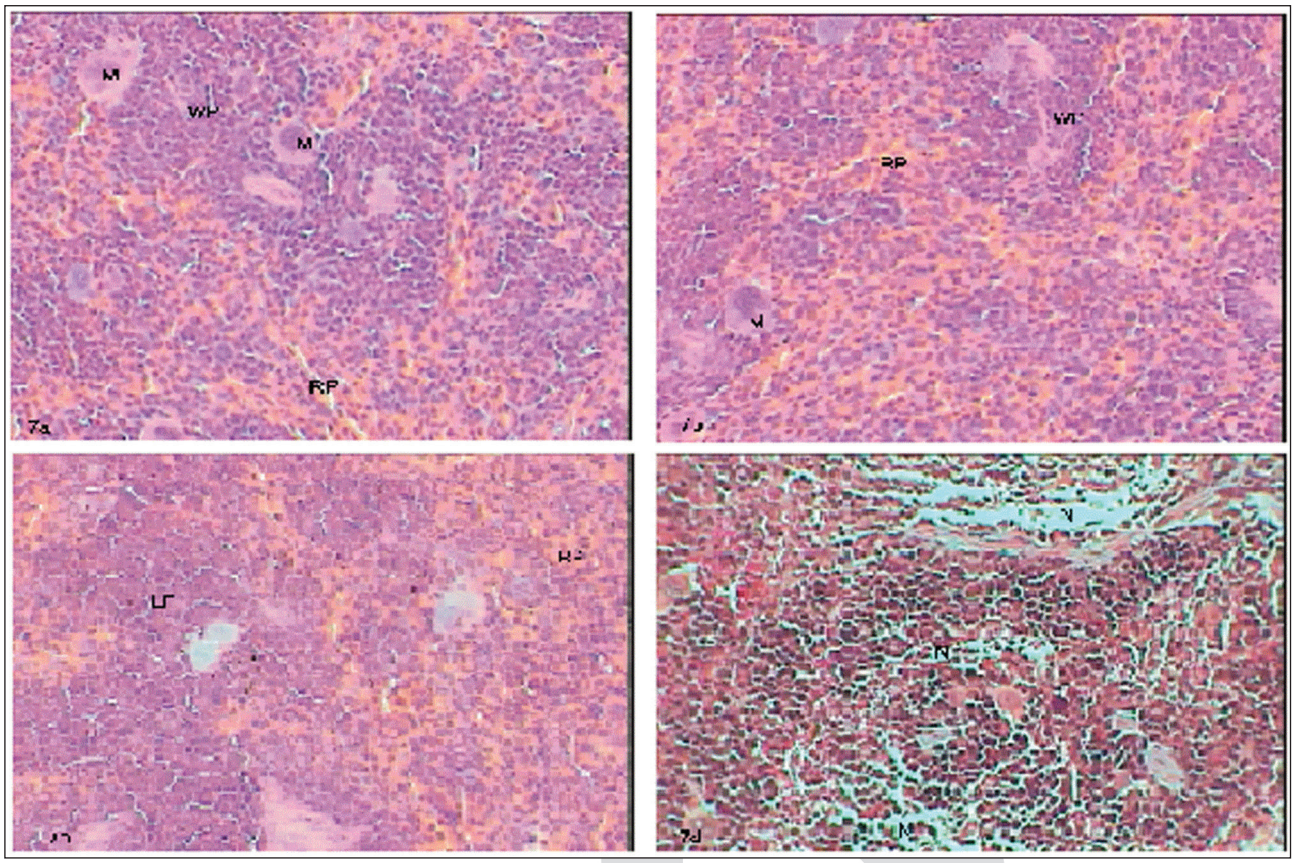


*Figure 7. Treated spleen sections with 2ml/kg/bw revealed more changes (7a mag.x400 H &E) distorted white pulp (WP), increasing number of macrophages (M), (7b mag.x400 H &E) small lymphoid follicle (WP), expansion of red pulp (RP) (7d mag.x400 H &E) abnormal white pulp (WP), congested red pulp (RP) (7d mag.x400 M.Tr.) necrotic foci (N).*

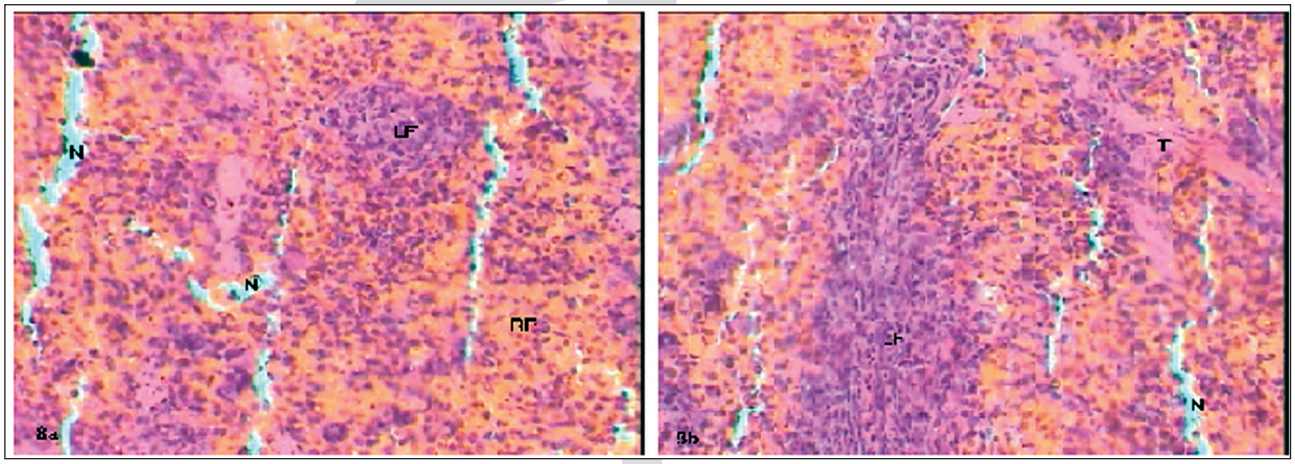


*Figure 8. Treated spleen sections with 6ml/kg/bw of ethanol showed severe alterations (8a mag.x400 H &E) severe congestion in red pulp (RP), shrunken lymphoid follicle (LF), necrosis (N) (8b mag. x400 H&E) distorted lymphoid follicle(LF), necrosis (N), trabeculae (T).*

## References


1. *Becker U, Deis A, Sorensen TI, Gronbaek M, Borch-Johnsen K, et al. Prediction of risk of liver disease by alcohol intake, sex and age: A prospective population study. Hepatology, 1996; 23: 1025-1029.*

2. *Berg JM, Tymoczko JLeof, Stryer L. Ethanol Alters Energy Metabolism in the Liver. Biochemistry. 5th edition. 2002.*

3. *Brozoska M, Moniuszko J, Marcinkiewicz B, Sawicki B. Liver and kidney function and histology in rats exposed to cadmium and ethanol. Alcohol Alcohol 2003; 38: 2-10.*

4. *Bujanda L, Gutiérrez-Stampa MA, Marimón JM. El vino a dosis moderadas; salud o enfermedad. Med Clin (Barc) 1999; 112: 29-35.*

5. *Gouillon Z, Lucas D, Li J, Hagbjork A, French B, et al. Inhibition of Ethanol-Induced Liver Disease in the Intragastric Feeding Rat Model by Chlormethiazole. Exp Biol Med. 2000; 224(4), 302-308.*

6. *Harrisond J, Burt D. Pathology of alcoholic liver disease. Bailliére's Clin Gastroenterol 1993; 7: 641 -662.*

7. *Ismail M, Riely C. Alcoholic Fatty Liver. emedicine from WebMD. October 31, 2006.*

8. *Ito T, Molina M, Andriolo A, Borges R. The combination of atorvastatin and ethanol is not more hepatotoxic to rats than the administration of each drug alone. Br J Med Biol Res 2007; 40: 343-348.*

9. *Fairbanks K, Alcoholic Liver Disease. Disease Management FProject. The Cleveland Clinic, December 13, 2004.*

10. *Lau A, von D. V, Sander M, MacGuill M, Lanzke N, Spies C. Alcohol use disorder and perioperative immune dysfunction. Anesth. Analg. 2009; 108: 916-920*

11. *Nath B, Szabo G. Alcohol-induced modulation of signaling pathways in liver parenchymal and nonparenchymal cells: implications for immunity. Semin. Liver Dis. 2009; 29: 166-177.*

12. *Nava-Aguilera E, Andersson N, Harris E, Mitchell S, Hamel C, et al. Risk factors associated with recent transmissionof tuberculosis: systematic review and meta-analysis. Int. J Tuberc. Lung Dis. 2009; 13: 17-26.*

13. *Mann RE, Smart RG, Govoni R. The Epidemiology of Alcoholic Liver Disease. National Institute on Alcohol Abuse and Alcoholism Publication. September 29, 2004.*

14. *Rodés J, Urbano-Márquez A, Bach L. Alcohol y enfermedad. Barcelona, Prous JR 1990.*

15. *Roselle G. Alcohol and the immune system. Alcohol Health & Research World. 1992.*

16. *Rottenberg H. Liver Cell Membrane Adaptation to Chronic Alcohol Consumption . Liver pathology and Alcohol. Drug and Alcohol Reviews. 1992; Volume 2: 91-115, DOI: 10.1007/978-1-4612-0421-3_3*

17. *Szabo G, Mandrekar P. A recent perspective on alcohol, immunity, and host defense. Alcohol Clin Exp. 2009; Res 33: 220-232.*


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# Long-term effects of comprehensive interventional therapy on extremity osteosarcoma

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## Abstract

**Objective:** To analyze the effects of comprehensive interventional therapy on extremity osteosarcoma.

**Methods:** 65 cases of pathologically diagnosed extremity osteosarcoma were subjected to 3 treatment stages: 1) 2 treatment courses of regional intraarterial perfusion and systemic chemotherapy; 2) simultaneous low-dose regional intraarterial perfusion and radiotherapy; 3) 6 treatment courses of systemic chemotherapy. All the patients were followed up for 12-120 months. The cases were divided into 3 groups according to the stages (Group A: all three stages completed; Group B: the first two stages completed; Group C: only the first stage completed). The local tumor control rates, the cumulative survival rates and the median survival time of the three patient groups were retrospectively analyzed.

**Results:** The overall local control rate and local recurrence rate of Group A and Group B were 84.78% and 15.22%, respectively. The 1-, 3- and 5-year cumulative survival rates of Group A were 100.00%, 72.36% and 51.23%, respectively, and those of Group B were 80.28%, 7.31% and 7.32%, respectively. The 1- and 2-year cumulative survival rates of Group C were 28.31% and 0.00%, respectively. The median survival time values of the three groups all differed significantly (Group A and B: $X^2$=27.86, P < 0.0001; Group A and C: $X^2$= 54.05, P<0.0001; Group B and C: $X^2$=17.06, P<0.0001).

**Conclusions:** The comprehensive interventional therapy effectively led to satisfactory local tumor control rates and long-term survival rates.

**Key words:** Osteosarcoma, radiology, intervention, radiotherapy, comprehensive therapy, evaluation.

## Introduction

The 5-year survival rate of extremity osteosarcoma has been elevated from 25% to approximately 85% since the promotion of auxiliary chemotherapy in the 1970s[1]. Particularly, limb salvage surgeries have been widely applied owing to the increased tumor necrosis rate from 65% to 100% after combining intraarterial perfusion[2]. The 2-year postoperative mortality rate of individual chemotherapy or radiotherapy is 80% due to the subclinical metastasis after amputation. The long-term survival is most significantly affected by pulmonary metastasis[3]. Actually, the osteosarcoma patients may have suffered from subclinical metastasis upon the first visit, which determines the long-term treatment efficacy.

Combining intraarterial chemotherapy with radiotherapy is the fundamental and primary therapy [4]. However, limb salvage surgeries are limited by the following issues: 1) considerable metastasis rate and local recurrence rate; 2) the inevitably disability that prevents patients from agreeing to surgeries[5]. 83 cases of patients who refused to receive surgical operations from March 2002 to January 2012 were subjected to comprehensive interventional therapy, in which 65 cases with complete medical records were followed up for a long time.

## Materials and Methods

### *Clinical data*

The 65 cases who were pathologically diagnoses as extremity osteosarcoma aging 6-71 years old (median age: 19.13) consisted of 30 males and 35 females. Position: 20 cases of femur, 20 cases of tibiofibula, 9 cases of pelvis, and 7 cases of humerus. Enneking staging: 2 cases of stage IIA, 55

cases of stage IIB, and 8 cases of stage III. X-ray typing: 5 cases of sclerotic type, 4 cases of osteolytic type, and 56 cases of mixed type.

***Treatment methods (3 stages)***

Stage 1: 45 mg/m$^2$ adriamycin (administered in 2 days) and 95 mg/m$^2$ cisplatin (administered in 3 days) were intraductally perfused by femoral artery intubation or inlying catheter (distal end of the catheter was placed above tumor blood supply). The drugs were perfused by a constant speed pump in 30 min. In the late stage, 13 patients were simultaneously intravenously administered with 10 g/m$^2$ ifosfamide (administered in 5 days). The treatment consisted of 2 courses with 4 weeks of interval.

Stage 2: Radiotherapy after the enhanced sensitivity by low-dose regional intraarterial perfusion. The patients were irradiated by photon beam. Dose at the tumor position: 70-90 Gy (30-40 times), dose at the proximal tumor position: 60 Gy (30 times), dose at the distal tumor position: 40 Gy (20 times). The irradiation consisted of 2 courses with 4 weeks of break. At the beginning of each course, 10-20 mg cisplatin and 10 mg adriamycin were continuously perfused by the inlying catheter for 10 days and 5 days, respectively.

Stage 3: The patients were subjected to systemic chemotherapy with the combination of adriamycin, cisplatin and ifosfamide, or high-dose methopterin, or the alternated administration of etoposide and catharanthine.

***Observation index***

The patients were followed up by observing the changes of symptoms and signs, the recovery of limb functions and the side effects after treatment. The regional tumor control was evaluated by regional angiography and X-ray plain films. The tumor recurrence and metastasis as well as patient survival rate were recorded. Since there are no uniform standards evaluating the regional tumor control, the control situation was assessed according to the disappeared symptoms and signs, the recovered limb functions, the region angiography indicating tumor pathological blood vessel vanishing, and the clearly defined newly formed bones instead of soft tissue lumps indicated by X-ray plain films.

***Statistical analysis***

The data were analyzed according to the finished stages. The cumulative survival rate at an indicated time was evaluated by the life table method, and the median survival time was evaluated and the survival curves were compared by the Log-Rank detection. All the data were statistically analyzed by SPSS13.0.

## Results

***Treatment and follow-up***

100% of the patients were followed up for 12-120 months (from March 2002 to January 2012). The 65 cases were divided into three groups (Group A: 35 cases, 3 stages completed; Group B: 17 cases, the first 2 stages completed; Group C: 13 cases, the first stage completed).

***Regional tumor control***

The pains and limb functions of 46 cases undergoing regional chemotherapy intervention and radiotherapy were mitigated and recovered, respectively. The angiography and the X-ray plain film show that the blood transport to tumor was gradually deactivated. The soft tissue lumps were replaced with new bones, yielding sharply bordered bony shells that shrank during the follow-up (Figure 1-5). The regional control rate and the regional recurrence rate were 84.78% (39/46) and 15.22% (7/46), respectively. In contrast, the regional focuses of all patients in Group C were uncontrollable.

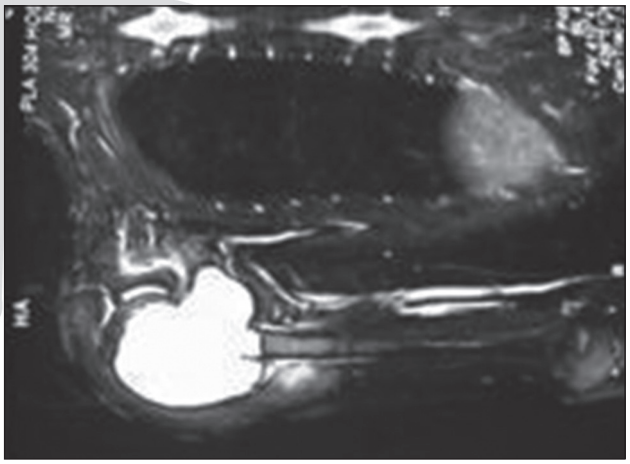

*Figure 1. Osteosarcoma of the right humerus. The angiography exhibits active blood transport, and the axillary arteries were oppressed and interrupted soft tissue lumps. Besides, the radial and brachial artery pulses disappeared.*

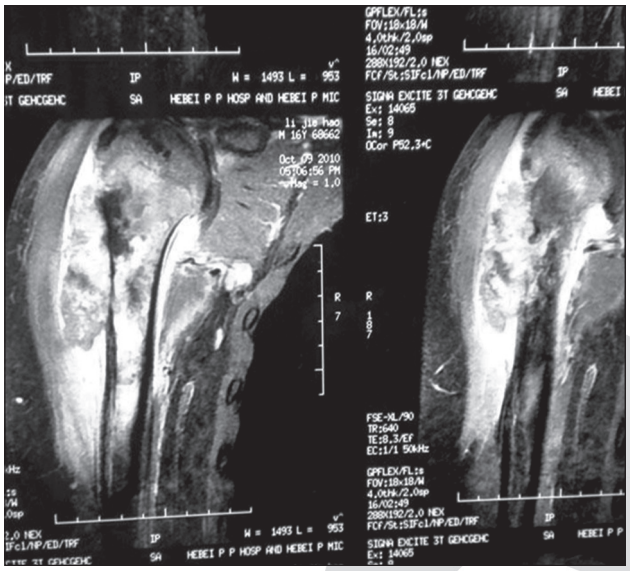


*Figure 2. The same patient as that in Figure 1. The follow-up examination before the second perfusion chemotherapy shows that the tumor blood supply was significantly reduced, the soft tissues shrank, and the brachial arteries were unclogged.*

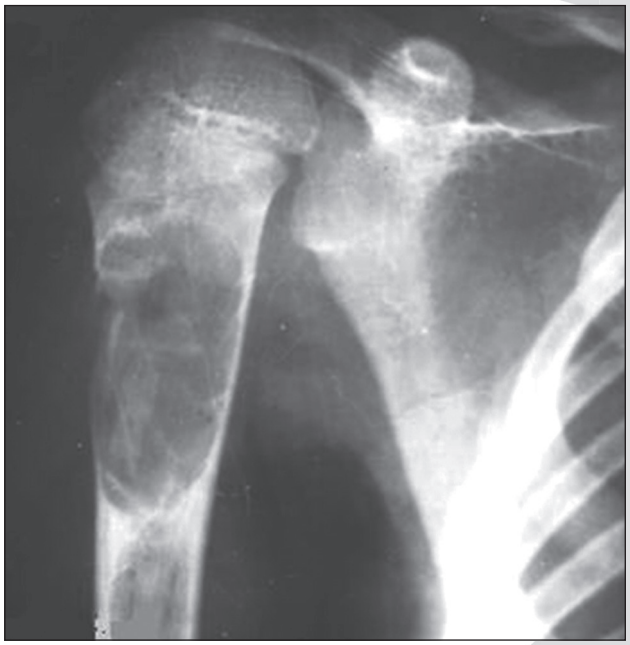

*Figure 3. X-ray plain film of the patient who had been followed up for 12 months after regional intervention + radiotherapy. The soft tissue lumps were replaced with new bones, yielding sharply bordered bony shells.*

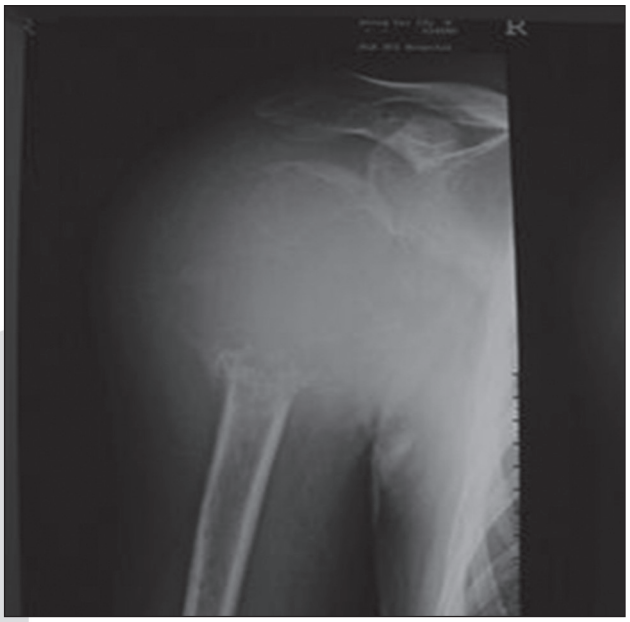

*Figure 4. The same patient as that in Figure 3. X-ray plain film of the patient who had been followed up for 24 months. The bony shells shrank.*

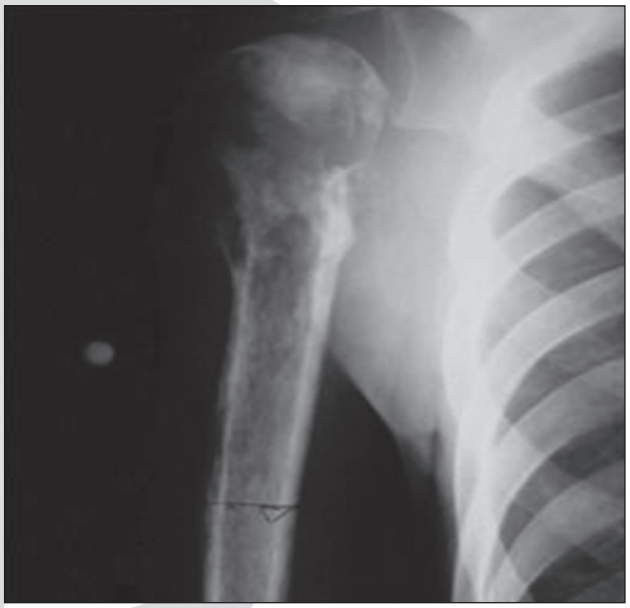

*Figure 5. The same patient as that in Figure 3. X-ray plain film of the patient who had been followed up for 36 months. The bony shells shrank continuously.*

***Survival***

The 1-, 3- and 5-year cumulative survival rates of Group A were 100.00%, 72.36% and 51.23%, respectively, and those of Group B were 80.28%, 7.31% and 7.32%, respectively. The 1- and 2-year cumulative survival rates of Group C were 28.31% and 0.00%, respectively. The median survival time values of the three groups all differed significantly

(Group A and B: $X^2$=27.86, P < 0.0001; Group A and C: $X^2$= 54.05, P<0.0001; Group B and C: $X^2$=17.06, P<0.0001) (Figure 6).

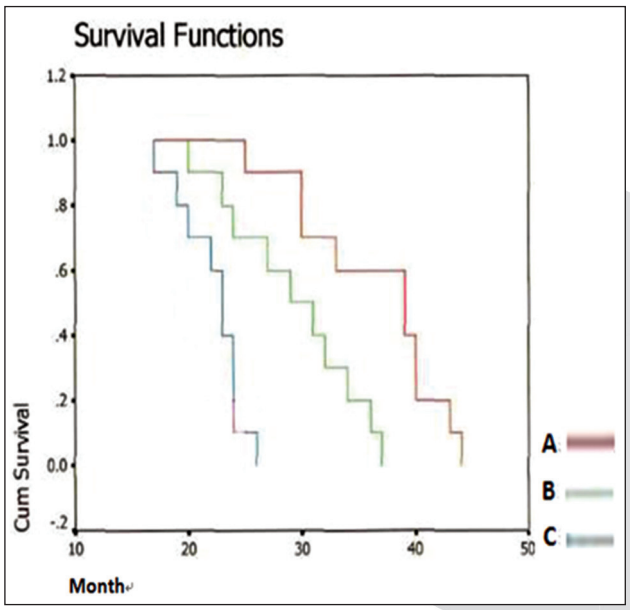


*Figure 6. Survival curves of the 3 groups of patients undergoing comprehensive interventional therapy.*

***Complications***

5 cases of early-stage arterial inlying catheter induced vascular intima injuries and thrombembolia. 13 cases of perfusion chemotherapy and 3 cases of tumor blood supply thrombembolia led to regional chemical dermatitis and regional skin ulcer necrosis, respectively. Long-term follow up, tumor-involving osteoepiphysis or joints, or ceased growth of adolescent osteoepiphysis after radiotherapy led to lameness and amyotrophy. The 10 cases of incomplete fracture owing to sterile necrosis did not recur in the follow up. 2 cases were developed into chronic osteomyelitis, which were subjected to amputation after 10 years of follow-up. The pathological examination did not indicate any tumor cells.

## Discussion

***Objective of comprehensive interventional therapy of extremity osteosarcoma***

Extremity osteosarcoma patients are mainly being treated with surgeries, which have been assisted with chemotherapy to boost the survival rate and limb functions. Especially, the histopathological tumor necrosis rate has reached up to 65%-100% owing to the intraarterial chemotherapy[6]. Combining intraarterial chemotherapy with radiotherapy is the fundamental and primary therapy nowadays. Nevertheless, limb salvage surgeries are limited by the following issues: 1) considerable metastasis rate and local recurrence rate; 2) the inevitably disability that prevents patients from agreeing to surgeries[7]. Thus, we attempted to treat the patients with comprehensive interventional therapy since 2002, aiming to find out a possible therapy that is readily acceptable and is able to prolong the survival time and improve the quality of life.

***Long-term treatment efficacy***

***Regional status***

7 IIB stage cases in Group A and B recurred, accounting for 15.22%. The other 39 cases were well controlled continuously. The recurrence rates of the patients receiving preoperative and postoperative chemotherapy after limb salvage ranged between 15%-25%, which may be associated with clinical staging and drug sensitivity, etc.[8, 9]. In this study, 1 case that recurred was subjected to amputation, and the pathological examination suggests only a small amount of tumor cells at bone edges. We hold that comprehensive interventional therapy and limb salvage are competitive in the regional tumor treatment. However, the unsatisfactory effects of this method on osteolytic type and/or pathological fracture patients require amputation. Of the 4 osteolytic type patients in Group C, 2 cases were further developed into joint effusion, infection and cachexia. The other 2 cases that were improved clinically suffered from increasingly aggravated pathological fracture.

***Survival***

The median survival time values of the three groups differed significantly (Group A: 47.31 months; Group B: 22.06 months; Group C: 7.95 months; P<0.0001). The 1-, 3-, 5- and 10-year cumulative survival rates of Group A were 100.00%, 72.36%, 51.23% and 11.98%, respectively, and the 1-, 3-, 5- and 6-year cumulative survival rates of Group B were 80.28%, 7.31%, 7.32% and 0.00%, respectively. The 1- and 2-year cumulative survival rates of Group C were 28.31% and 0.00%, respectively. It has been previously reported that the 5-year sur-

vival rates ranged between 13%-85% owing to the different sensitivities to chemotherapy drugs[10, 11]. The 5-year survival rate of Group A who had undergone all treatment stages was 52.23%, indicating that comprehensive interventional therapy is of long-term effects.

***Factors influencing the long-term survival***

Pulmonary metastasis mainly contributes to the failure of 40% of long-term survival[12-14]. The 5-year and 3-year metastasis mortality rates of Group A and Group B were 42.86% (15/35) and 88.24% (15/17) respectively, suggesting that the difference between the two groups was dominated by the systemic chemotherapy. Although Group A had been subjected to systemic chemotherapy, the inadequate experience and the insufficient dose and intensity of chemotherapy drugs resulted in approximately 24% metastasis-relating death annually within 3-10 years. It has been reported that 80% of the osteosarcoma patients suffered from pulmonary metastasis as well. In this study, 4 cases and 7 cases had suffered from pulmonary metastasis as soon as they visited the doctor and in their 1st and 2nd stages, respectively. Hence, the long-term treatment efficacy is predominated by devastating the early-stage subclinical metastasized focuses. The survival rates of 13 patients could be further elevated by combining the regional arterial chemotherapy with intravenous one. The gene expression studies on osteosarcoma in recent years have revealed the factors relevant to prognosis. The long-term survival rate of osteosarcoma can be increased by non-cross-resistant drugs and reverse-coding genetic drugs[15-18].

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**References**

1. *Quintana J, Beresi V, DelPozo H. Intra-arterial cisplatin given prior to surgery in osteosarcoma: Grade of necrosis and size of tumor as major prognostic factors. Am J Pediatr Hematol Onco, 1991; 13(3): 269-273.*
2. *Ratnagiri R, Garg V, Chaturvedi R. Extra osseous osteosarcoma of the retroperitoneum: An unusual entity. J Cancer Res Ther, 2012; 8(3): 424-426.*
3. *Matthews E, Snell K, Coats H. Intra-arterial chemotherapy for limb preservation in patients with osteosarcoma: Nursing implications. Clin J Oncol Nurs, 2006; 10(5): 581-589.*
4. *Zhang HJ, Yang JJ, Lu JP, et al. Use of intra-arterial chemotherapy and embolization before limb salvage surgery for osteosarcoma of the lower extremity. Cardiovasc Intervent Radiol, 2009; 32(4): 672-678.*
5. *Li D, Cui Q, Liu Y, et al. Chemotherapy response analysis for osteosarcom with intra-arterial chemotherapy by subcutaneous implantable delivery system. Pathol Oncol Res, 2011; 17(4): 947-953.*
6. *Hughes D. Strategies for the targeted delivery of therapeutics for osteosarcoma. Expert Opin Drug Deliv, 2009; 6(12): 1311-1321.*
7. *Anderson PM, Pearson M. Novel therapeutic approaches in pediatric and young adult sarcomas. Curr Oncol Rep, 2006; 8(4): 310-315.*
8. *Jaffe N. Osteosarcoma: Review of the past, impact on the future. The american experience. Cancer Treat Res, 2009; 152: 239-262.*
9. *Mir O, Ropert S, Babinet A, et al. Hyper-alkalinization without hyper-hydration for the prevention of high-dose methotrexate acute nephrotoxicity in patients with osteosarcoma. Cancer Chemother Pharmacol, 2010; 66(6): 1059-1063.*
10. *Cui Q, Li DF, Liu C, et al. Two case-reports of the limb salvage treatment of osteosarcoma consolidated with obvious pathological fractures. Pathol Oncol Res, 2011; 17(4): 973-979.*
11. *Woźniak W, Rychlowska M, Izbicki T, et al. Results of treatment in osteosarcoma--chemotherapy with adm and cddp. Med Wieku Rozwoj, 2000; 4(1 Suppl 2): 97-102.*
12. *Liu ZL, Wang G, Peng AF, et al. Fatty acid synthase expression in osteosarcoma and its correlation with pulmonary metastasis. Oncol Lett, 2012; 4(5): 878-882.*

13. *Hameed S, Vijayan S, Naik AM, et al. Multicentric osteosarcoma. Singapore Med J, 2012; 53(10): e214-7.*

14. *Li Y, Liao Q, Li K, et al. Knockdown of endothelin a receptor expression inhibits osteosarcoma pulmonary metastasis in an orthotopic xenograft mouse model. Mol Med Report, 2012; 5(6): 1391-1395.*

15. *Toguchida J, Ishizaki K, Sasaki MS, et al. Chromosomal reorganization for the expression of recessive mutation of retinoblastoma susceptibility gene in the development of osteosarcoma. Cancer Res, 1988; 48(14): 3939-3943.*

16. *Feugeas O, Guriec N, Babin-Boilletot A, et al. Loss of heterozygosity of the rb gene is a poor prognostic factor in patients with osteosarcoma. J Clin Oncol, 1996; 14(2): 467-472.*

17. *Miller CW, Aslo A, Won A, et al. Alterations of the p53, rb and mdm2 genes in osteosarcoma. J Cancer Res Clin Oncol, 1996; 122(9): 559-565.*

18. *Flowers S, Beck GR Jr, Moran E. Transcriptional activation by prb and its coordination with swi/snf recruitment. Cancer Res, 2010; 70(21): 8282-8287*

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# The effect of aerobic and anaerobic swimming exercises on mda, sod and gsh levels of elite swimmers

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**Abstract**

**Backround:** In recent years there has been an extensive research on the effect of exercise upon the oxidative stress and the anti-oxidant capacity. Most of these studies emphasize that the physical activity promotes the oxidative stress by increasing the free radical concentration in various tissues and affects the antioxidant defense mechanism. The increase in oxygen free radicals was reported to cause protein, fat and DNA damage.

**Purpose:** This study was carried out to test the hypothesis that different types of exercise (aerobic and anaerobic) would have a different effect on the MDA, SOD and GSH values of young elite swimmers.

**Method & materials:** The study was participated by 24 elite woman swimmers with ages of 18-21 years, heights of 161-170 cm, body weights of 61-64 kg and Body Mass Index (BMI) 21-23 kg/m$^2$.

**Results:** It was observed that the single anaerobic swimming exercise caused a statistically significant increase in MDA concentration and a statistically significant decrease in GSH and SOD activities. At the end of the study after six week it was observed that the MDA values of AG swimmers showed a statistically significant decrease and their GSH and SOD activities showed a statistically significant increase.

**Conclusion:** In this study it was also found that the anaerobic swimming exercise increased the MDA and decreased the SOD and GSH enzyme activity. The effect of the aerobic exercise was found to be opposite. The MDA concentration decreased and the SOD and GSH activities increased.

**Key words:** Swimming, oxidant, aerobic, superoxide dismutase.

## Introduction

Swimming is one of the most common sports performed on both physical activity and competitive basis all over the world. There are four different swimming styles as a competitive sport namely freestyle, breaststroke, butterfly and backstroke. The energy needed during the swimming activity is provided by two different ways. The first one is through the anaerobic, phosphocreatine (PC) or glycolysis and the second way is the electron transfer system from the aerobic mechanism (1). There are some studies in the literature reporting that the swimming exercise increases to oxidative stress and decreases the antioxidant capacity in both to children and the young people (1,2). In recent years there has been an extensive research on the effect of exercise upon the oxidative stress and the anti-oxidant capacity (3-6). Most of these studies emphasize that the physical activity promotes the oxidative stress by increasing the free radical concentration in various tissues and affects the antioxidant defense mechanism (7-11). The increase in oxygen free radicals was reported to cause protein, fat and DNA damage (12,13). It was also emphasized that acute exercise increases the malondialdehyde (MDA) concentration (14,15) and decreases the activity of anti-oxidant enzyme such as glutathione (GSH) and superoxide dismutase (SOD, 16-19). On the other hand there are numerous studies reported that the regular aerobic exercise decreases MDA values (20,21) and increases the GSH and SOD activity (22-24).This study was carried out to test the hypothesis that different types of exercise (aerobic and anaerobic) would have a different effect on the MDA, SOD and GSH values of young elite swimmers.

## Materials and Method

### *Participants*

The study was participated by 24 elite female swimmers with the age of 18-21 years who had been doing swimming in last five years. The participants were adequately briefed about risks and importance of the study and all the participants signed a consent form prior to the study. The study was also approved by the ethical committee of Inonu University (Protocol No: 2010/125). The participants were chosen according to the following criteria a being a non-smoker and non-drinker b) having no apparent health defect c) using no medication or antioxidant supplementation such as vitamins and (d) having no skeletal muscle disease. The participants were arbitrarily divided into three groups as follows: a) Control group (CG, n=8), (b) aerobic group (AG, n=8) and (c) anaerobic group (ANG, n=8).

### *Exercise protocol*

Aerobic exercises: these are 2-3 minutes exercises which are based upon the reaction where glycogen is converted into energy by a charge (electron) transfer reaction. Anaerobic exercises on the other hand take 2 minutes or less where energy is supplied by PC, Gylocolysis or LA. In this context the group ANG was subjected to 50 meter freestyle swimming (30s) while the group AG had to swim 800m (12 minute) for six weeks and three days a week (Tuesday, Thursday and Saturday)

### *Collecting the blood samples*

There were 10 cc venous blood samples taken from the left arms of the participants at sublime (sitting) position using syringes with plastic tips before and after the experiments. The samples were immediately centrifuged and kept at the deep freeze a -80°C until the final analysis.

### *The determination of the biochemical parameters*

The level of MDA a product of the lipid peroxidation was measured spectrophotometrically by the absorption of the pink-red color at 532 nm formed as result of the reaction with TBA at 95°C. Plasma MDA level was measured by the use of Uchiyama and Mihara method (25) The sample absorbance was first multiplied with a dilution factor of 10 and then with a factor obtained from the standard graph which gave the amount of MDA in nmol/l. GSH determination was made spectrophotometricaly by monitoring the peak at 410 nm corresponding to the product formed by the reaction of the Elman reagent with sulphydrile groups using the method developed by Fairbanks and Klee (26). Then the absorbance of the samples was multiplied by the factor obtained from which give the activity of GSH in μmol/l. Total (Cu–Zn and Mn) SOD (EC 1.15.1.1) determination is based upon the conversion of the super oxide radicals generated by xsantine oxidase into $H_2O_2$ which reduces nitroblue tetrazolium (NBT) into a blue colored formazan which gives an absorbance peak at 560 nm (27). The data are given in U/l.

All the statistical data are given as mean ± Sd I this study. The data analyses we started with the homogeneity test. Since the data do not show a homogeneous distribution the statistical significance levels of the pre and post- exercise values were determined by "Two Related Samples Tests". All the statistical analysis were carried out with 17.0 SPSS (Statistical Package for Social Science, SPSS Inc.) software.

## Results

The study was participated by 24 elite woman swimmers with ages of 18-21 years, heights of 161-170 cm, body weights of 61-64 kg and Body Mass Index (BMI) 21-23 kg/m$^2$ (Table 1). There are no statistical differences between any groups as regards to these parameters.

The pre and post exercise MDA, GSH and SOD parameters of ANG swimmers showed a statistically significant differences ($p<0.01$). CG and AG swimmers on the other hand showed no statically significant difference in these biochemical parameters before and after the exercise ($p>0.01$).

However the MDA values of ANG swimmer showed a statistically significant increase and their GSH and SOD a statistically significant decrease after the exercise (Table 2).

When we examine the pre and post exercise values of the MDA values of the participants we observe that there was a statistically significant increase in ANG swimmers (p=.000) and there were no such changes CG and AG swimmers (p=.250 and p=.843 respectively). This was the case for

*Table 1. Some physical parameters of the participants*

| Parameters (n=8) | CG | ANG | AG |
|---|---|---|---|
| | X±Sd | X±Sd | X±Sd |
| **Age** (years) | 19.54±2.52 | 20.05±2.80 | 20.20±2.10 |
| **Height** (cm) | 164.50±6.60 | 166.15±5.80 | 165.80±4.40 |
| **Body weight** (kg) | 60.25±4.63 | 61.10±3.95 | 59.50±4.80 |
| **Body mass index**(BMI, kg/m$^2$) | 22.45±1.25 | 22.00±1.69 | 22.10±1.10 |

*Table 2. Pre- and Post-exercise MDA, GSH and SOD analyses*

| Parameters | n | CG X±Sd | p | ANG X±Sd | p | AG X±Sd | p |
|---|---|---|---|---|---|---|---|
| | | 8 | | 8 | | 8 | |
| MDA (nmol/l) | Pre-test | 25.034.17± | .843 | 26.903.10± | .000* | 24.704.00± | .250 |
| | Post-test | 25.265.98± | | 31.654.50± | | 25.324.32± | |
| GSH (µmol/l) | Pre-test | 1.39±.071 | .719 | 1.40±.069 | .000* | 1.56±.074 | .503 |
| | Post-test | 1.41±.063 | | 1.16±.075 | | 1.50±.082 | |
| SOD (U/l) | Pre-test | 1.81±.084 | .914 | 1.38±.078 | .000* | 1.72±.067 | .741 |
| | Post-test | 1.82±.087 | | 1.20±.082 | | 1.75±.062 | |

*Table 3. Pre-test and MDA, GSH and SOD Analyses after six weeks*

| Parameters | n | CG X±Sd | p | ANG X±Sd | p | AG X±Sd | p |
|---|---|---|---|---|---|---|---|
| | | 8 | | 8 | | 8 | |
| MDA (nmol/l) | Pre-test | 24.80±5.72 | .785 | 25.42±4.05 | .031* | 25.12±3.75 | .037* |
| | Post-test | 25.43±5.13 | | 30.90±3.85 | | 22.45±4.23 | |
| GSH (µmol/l) | Pre-test | 1.45±.085 | .542 | 1.46±.074 | .021* | 1.51±.069 | .014* |
| | Post-test | 1.49±.073 | | 1.05±.081 | | 1.80±.049 | |
| SOD (U/l) | Pre-test | 1.73±.076 | .953 | 1.41±.064 | .017* | 1.68±.067 | .019* |
| | Post-test | 1.74±.082 | | 1.12±.052 | | 1.96±.054 | |

*Table 4. MDA, GSH and SOD 1st and 2nd Pre-test comparison*

| Parameters | n | CG X±Sd | p | ANG X±Sd | P | AG X±Sd | p |
|---|---|---|---|---|---|---|---|
| | | 8 | | 8 | | 8 | |
| MDA (nmol/l) | 1st. Pre-test | 25.03±4.17 | .812 | 26.90±3.10 | .162 | 24.70±4.00 | .830 |
| | 2nd. Pre-test | 24.80±5.72 | | 25.42±4.05 | | 25.12±3.75 | |
| GSH (µmol/l) | 1st. Pre-test | 1.39±.071 | .417 | 1.40±.069 | .420 | 1.56±.074 | .690 |
| | 2nd. Pre-test | 1.45±.085 | | 1.46±.074 | | 1.51±.069 | |
| SOD (U/l) | 1st. Pre-test | 1.81±.084 | .281 | 1.38±.078 | .784 | 1.72±.067 | .831 |
| | 2nd. Pre-test | 1.73±.076 | | 1.41±.064 | | 1.68±.067 | |

both GSH and SOD values which showed statistically significant decrease in ANG swimmers (p=.000). However both CG and AG swimmers showed no statistically significant changes in neither GSH (p=.719 and p=.503 respectively) nor SOD (p=.914 and p=.741 respectively) activities.

After six weeks there was a statistically significant increase in MDA values of ANG swimmers (p=.031) while there was a statistically significant decrease in the corresponding values of AG swimmers (p=.037) six weeks after the exercises. The same trend was reverse for GSH enzyme activity. It showed a statistically significant decrease in ANG swimmers (p=.021) and statistically significant increase for AG swimmer (p=.014) after six weeks of exercise. The very same trend was the case for SOD

*Table 5. MDA, GSH and SOD 1st and 2nd Post-test comparison*

| Parameters | n | CG X±Sd | p | ANG X±Sd | p | AG X±Sd | p |
|---|---|---|---|---|---|---|---|
| | | 8 | | 8 | | 8 | |
| MDA (nmol/l) | 1st Post-test | 25.26±5.98 | .904 | 31.65±4.50 | .826 | 25.32±4.32 | .004* |
| | 2nd Post-test | 25.43±5.13 | | 30.90±3.85 | | 22.45±4.23 | |
| GSH (µmol/l) | 1st Post-test | 1.41±.063 | .210 | 1.16±.075 | .097 | 1.50±.082 | .000* |
| | 2nd Post-test | 1.49±.073 | | 1.05±.081 | | 1.80±.049 | |
| SOD (U/l) | 1st Post-test | 1.82±.087 | .052 | 1.20±.082 | .174 | 1.75±.062 | .013* |
| | 2nd Post-test | 1.74±.082 | | 1.12±.052 | | 1.96±.054 | |

values. According to that there was a statistically significant decrease in the SOD values of ANG swimmers (p=.017) and there was a statistically significant increase in the corresponding values of AG swimmers after six weeks of exercise (p=.019). In CG swimmers there was not any statistically significant change in any of these parameters.

It was observed that there was no statistically significant difference between the pre test values taken at the first and the sixth week of the study in MDA, GSH and SOD parameters in none of the groups (p>0.05)

There was a statistically significant difference between the MDA values of AG swimmers recorded after the first training at the first week and the final training at the sixth weeks (p=.004). The GSH and SOD enzyme activities of AG swimmers were observed to show statistically significant increase after six weeks compared with the first post exercise values (p=.000, p=.013 respectively). However there was no statistically significant difference between the corresponding values of both the CG and ANG swimmers.

## Discussion

In this study where the effect of aerobic and anaerobic swimming exercises upon MDA, GSH and SOD parameters were investigated. It was observed that the single anaerobic swimming exercise caused a statistically significant increase in MDA concentration and a statistically significant decrease in GSH and SOD activities. On the other hand the application of single aerobic exercise was found to cause no statistically significant change in either MDA concentration or GSH and SOD activities. At the end of the study after six week it was observed that the MDA values of AG swimmers showed a statistically significant decrease and their GSH and SOD activities showed a statistically significant increase. However in ANG swimmers the plasma MDA values had a statistically significant increase while their GSH and SOD values had a statistically significant decrease in the same period.

The comparison of the pre and post exercise values of all the groups showed that especially in AG swimmers there was a statistically significant difference MDA, GSH and SOD values after the exercise. There were no statistically significant changes in the corresponding values of CG and ANG swimmers.

MDA is a marker which shows the free radical activity in lipid peroxidation and cell membranes (28,29). The excess generation of free radicals in muscles as result of strenuous exercise causes oxidative damage (30). There are numerous articles in the literature which report that acute exercises increase the MDA value. It was reported that acute exercise (14), exhaustive cycling exercise (15), submaximal exercise (31) and medium intensity anaerobic exercise (32) increased the MDA value. These results are parallel with the results observed in this study in the MDA values of ANG swimmers. However there is no consensus on the effect of single anaerobic exercise, since there are articles reporting that it did not change (20,34) or decreased (35,36) the MDA values.

In the current study it was observed that the MDA value of AG swimmers showed a decrease after six weeks of exercise. This outcome is in good accordance with the studies of Miyazaki et.al, (21) and Leaf et.al, (20) where they reported that regular aerobic exercise decreased MDA values. Both the literature data and the results of this study showed that aerobic exercises are much

beneficial on the skeleton muscles than the anaerobic exercises.

The antioxidant defense mechanism starts to generate enzymatic antioxidants such as GSH and SOD in order to prevent the destructive effect of excess free radical formation (24,37). Deminice et.al, (38) reported that the acute endurance exercise and Berzosa et.al, (17) claimed that the anaerobic cycling exercise or single exercises decreased GSH enzyme activity. These results are in compliance with our findings. In this study it was found that the anaerobic exercises applied to the ANG swimmers for six weeks caused a statistically significant decrease in GSH value. On the other hand the application of aerobic exercise to the AG swimmers for the same period caused a statistically significant increase in GSH activity. These results are parallel with some studies in the literature (19,23,24). The enzymatic SOD activity of ANG swimmers also displayed a statistically significant decrease after six week of anaerobic exercise. Shin et.al, (16) reported that there was a marked decrease in the SOD activity after the application of 6-week anaerobic program. Similarly Karabulut et.al, (39) and Berzosa et.al, (17) respectively reported that the single acute and anaerobic cycling exercise decreased the SOD activity by a statistically significant extent. On the other hand the six week aerobic swimming exercise resulted in a statistically significant increase in SOD enzyme activity. These results are in good accordance with some of the literature data (22-24).

In conclusion there is a consensus in the literature on the fact that acute anaerobic exercises adversely affect the MDA, GSH and SOD values. But the beneficial effects of the regular aerobic exercises have been mentioned in many studies. In this study it was also found that the anaerobic swimming exercise increased the MDA and decreased the SOD and GSH enzyme activity. The effect of the aerobic exercise was found to be opposite. The MDA concentration decreased and the SOD and GSH activities increased. However the studies related to swimming exercises is limited and there is urgent need of further studies in this area.

## References


1. *Leelarungrayub D, Sawattikanon N, Klaphajone J, Pothongsunan P and Bloomer JR. Coenzyme Q10 supplementation decreases oxidative stress and improves physical performance in young swimmers: A pilot study. The Open Sports Medicine Journal 2010; 4: 1-8.*

2. *Gougoura S, Nilolaidis MG, Kostaropoulos IA, et al. Increased oxidative stress indices in the blood of child swimmers. Eur J Appl Physiol 2007; 100: 235-239.*

3. *Bloomer JR, and Fisher-Wellman HK. Blood oxidative stress biomarkers: influence of sex, exercise training status, and dietary intake. Gender Medicine 2008; 5(3): 218–228.*

4. *Jackson JM. Free radicals generated by contracting muscle: by-products of metabolism or key regulators of muscle function? Free Radical Biology and Medicine 2008; 44(2): 132–141.*

5. *Powers SK, and Jackson MJ. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiological Reviews 2008; 88(4): 1243–276.*

6. *Manna I, Jana K, and Samanta PK. Intensive swimming exercise–induced oxidative stress and reproductive dysfunction in male wistar rats: Protective role of alpha-tocopherol succinate. Can J Appl Physiol 2004; 29: 172–185.*

7. *Packer L. Oxidants, antioxidant nutrient and the athletes. J Sports Sci 1997; 15: 353–263.*

8. *Radak Z, Asano K, Inoue M, Kizaki T, Oh-Ishi S, Suzuki K, Taniguchi N, and Ohno H. Superoxide dismutase derivative reduces oxidative damage in skeletal muscle of rats during exhaustive exercise. J Appl Physiol 1995; 79: 129–135.*

9. *Lewandowski P, Hübner-Wozniac E. Effect of competitive pentathlon training on the antioxidant defense components. Biomedical Human Kinetics 2010; 2: 78-80.*

10. *Elahi MM, Kong YX, Matata BM. Oxidative stress as a mediator of cardiovascular disease. Oxid Med Cell Longev 2009; 2(5): 259-269*

11. *Essick EE, Sam F. Oxidative stress and autophagy in cardiac disease neurological disorders, aging and cancer. Oxid Med Cell Longev 2010; 3(3): 167-177.*

12. *Jenkins RR. Exercise and oxidative stress methodology: a critique. Am.J.Clin.Nutr 2000; 72: 670-674.*

13. *Codoner-Franch P, Tavarez-Alonso S, Murria-Estal R, Tortajada-Girbes M, SimoJorda R, Alonso-Iglesias E. Elevated advanced oxidation protein (AOPPs) indicate metabolic risk in severely obese children. Nutr Metab Cardiovasc Dis 2012; 22(3): 237-243.*

14. *Marzatico F, Pansarasa O, Bertorelli L, Somenzini L, Della Valle G. Blood free radical antioxidant enzymes and lipid peroxides following long-distance and lactacidemic performances in highly trained aerobic and sprint athletes. J Sports Med Phys Fitness 1997; 37(4): 235-239.*

15. *Aguilo A, Tauler P, Fuantespina E, Tur AJ, Cordova A, Pons A. Antioxidant response to oxidative stress induced by exhaustive exercise. Physiol and Behav 2005; 84(1): 1-7.*

16. *Shin YA, Lee JH, Song W, Jun TW. Exercise training improves the antioxidant enzyme activity with no changes of telomere length. Mech Ageing Dev 2008; 129(5): 254–260.*

17. *Berzosa C, Cebrián I, Fuentes-Broto L, Gómez-Trullén E, Piedrafita E, Martínez-Ballarín E, López-Pingarrón L, Reiter RJ, García JJ. Acute exercise increases plasma total antioxidant status and antioxidant enzyme activities in untrained men. J Biomed Biotechnol 2011; 10: 1155-1162.*

18. *Revan S, Balci SS, Pepe H, Kurtoğlu F, Erol AE, Akkuş H. Short duration exhaustive running exercise does not modify lipid hydroperoxide glutathione peroxidase and catalase. J Sports Med Phys Fitness 2010; 50(2): 235-240.*

19. *Fatouros IG, Jamurtas AZ, Villiotou V, Pouliopoulou S, Fotinakis P, Taxildaris K, Deliconstantinos G. Oxidative stress responses in older men during endurance training and detraining. Med Sci Sports Exerc 2004; 36(12): 2065-2072.*

20. *Leaf DA, Kleinman MT, Hamilton M, Barstow TJ. The effect of exercise intensity on lipid peroxidation, Med Sci Sports Exerc 1997; 29(8): 1036-1039.*

21. *Miyazaki H, Oh-ishi S, Ookawara T, Kizaki T, Toshinai K, Ha S, Haga S, Ji LL, Ohno H. Strenuous endurance training in humans reduces oxidative stress following exhausting exercise. Eur J Appl Physiol 2001; 84(1-2): 1-6.*

22. *Tauler P, Sureda A, Cases N, Aguilo A, Rodriguez-Marroyo JA, Villa G, Tur JA, Pons A. Increased lymphocyte antioxidant defences in response to exhaustive exercise do not prevent oxidative damage. J Nutr Biochem 2006; 17(10): 665-671.*

23. *Schippinger G, Wonisch W, Abuja PM, Fankhauser F, Winklhofer-Roob BM, Halwachs G. Lipid peroxidation and antioxidant status in professional American football players during competition. Euro J Clin Invest 2002; 32(9): 686-692.*

24. *Onur E, Kabaroğlu C, Günay O, Var A, Yılmaz O, Dündar P, Tikiz C, Güvenç Y, Yüksel H. The Benefical Effect of Physical Exercise on Antioxidant Status in Asthmatic Children. Allergol Immunopathol 2011; 39(2): 90-95.*

25. *Fairbanks V, Klee GG. Biochemical aspects of hematology. In: N. W. Tietz Editor Ttextbook of clinical chemistry. W. B. Saunders, Philadelphia; 1986. 1532-1534.*

26. *Sun Y, Larry W, Oberley W, Ving U. A simple method for clinical assay of superoxide dismutase. Clin. Chem 1988; 34: 497-500.*

27. *Uchiyama M, Mihara M. Determination of malonaldehyde precursor in tissue by thiobarbituric acid test. Anal Biochem 1978; 34: 271-278.*

28. *Bloomer, RJ, Fry, AC, Falvo, MJ, and Moore, CA. Protein carbonyls are acutely elevated following single set anaerobic exercise in resistance trained men. J Sci Med Sport 2007; 10: 411–417.*

29. *Dixon, CB, Robertson, RJ, Goss, FL, Timmer, JM, Nagle, EF, and Evans, RW. The effect of acute resistance exercise on serum malondialdehyde in resistance-trained and untrained collegiate men. J Strength Cond Res 2006; 20: 693–698.*

30. *McArdle, A, Pattwell, D, Vasilaki, A, Griffiths, RD. and Jackson, MJ. Contractile activity-induced oxidative stress: cellular origin and adaptive responses. American Journal of Physiology 2001; 280(3): 621–627.*

31. *Ramel A, Wagner KH, Elmadfa I. Plasma antioxidants and lipid oxidation after submaximal resistance exercise in men. Eur J Nutr 2004; 43: 2-6.*

32. *Antoncic-Svetina M, Sentija D, Cipak A, Milicic D, Meinitzer A, Tatzber F, Andrisic L, Zelzer S, Zarkovic N. Ergometry induces systemic oxidative stress in healty human subjects. Tohoku J Exp Med 2010; 221(1): 43-48.*

33. *Bloomer JR, Cole JB. Relationship between blood lactate and oxidative stress biomarkers following acute exercise. Open Sports Medicine Journal 2009; 3: 44-48.*

34. *Munoz-Marin D, Olcina G, Tımon MC, Caballero MJ, Maynar M. Effect of different exercise intensities on oxidative stress markers and antioxidant res-*

*ponse in trained cyclists. J Sports Med Phys Fitness 2010; 50(1): 93-98.*

35. *Joo M, Maehata E, Tetsuo A, Akiko I, Murai F, Noboru M. The relationship between exercise-induced oxidative stress and the menstrual cycle. Eur J Appl Physiol 2004; 93(1-2): 82-86.*

36. *Dixon CB, Robertson RJ, Goss FL, Timmer JM, Nagle E, Evans RW. Effect of resistance training status on free radical production and muscle damage following acute exercise. Med Sci Sports Exerc 2003; 35(5): 157-162.*

37. *Picado C, Deulofen R, Lleonart R, Agusti M, Mullol J, Quinto L, Torra M. Dietary micronutrients/antioxidants and their relationship with bronchial asthma severity. Allergy 2001; 56(1): 43-49.*

38. *Deminice R, Sicchieri T, Payao PO, Jardao AA. Blood and salivary oxidative stress biomarkers following an acute session of resistance exercise in humans. Int J Sports Med 2010; 31(9): 599-603.*

39. *Karabulut AB, Kafkas ME, Savaş S, Hazar M, Kiran TR. Effect of Exhaustive Exercise on Oxidative Stress and Adenosine Deaminase Activities in Women Compared to Men. Journal of US-China Medical Science 2011; 8(3): 150-155.*

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# Epidemiological characteristics and survival of laringeal cancer in the population of the Nishava District

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**Abstract**

**The objective** of the paper was to evaluate the survival of patients with laringeal cancer in Nishava District.

**Methods:** Published data from the Population Cancer Register of Serbia for the period 1999 to 2008. were used. Age-standardized incidence and mortality rates were calculated, according to the standard World population. The survival patterns were estimated by Kaplan-Meier method and the long rank test was applied to test the differences in survival. Cox hazard model was also used.

**Results:** In the observed period, the average annual-standardized incidence rate was 75.39 (145.77 in males and 11.16 in females). The average annual-standardized mortality rate was 15.14 (29.67 in males and 0.17 in females). The majority of patients were male (93% vs 7%). The average age of patients was similar (men: 61.7±10.97 vs women: 61.8±12.3). In 204 (80.3%) patients the histological type of cancer was squamous cell carcinoma. The survival rate after first year was 70.2%, after second 55.2%, after third 51.3% and after fourth year the survival rate was 47.6%. The 5-year survival rate was 38.4%. The mean observed survival time was 35.1(95%; 31.876-38.369) months and median was 40.8(95%, 23.054-58.546).

**Conclusion:** An increasing incidence and mortality trend was found among patients with laringeal cancer in population of Nishava District. Laringeal cancer is curable disease but survival is lower than in countries in surounding because of high degree of cancer and decrease possibilities for curable therapy.

**Key words:** Incidence, laringeal cancer, mortality, survival.

## Introduction

Laryngeal cancer is the second most common malignancy of head and neck (1). Increasing incidence trends are seen in Central and Eastern Europe and in the most developing countries, while in North America and Western Europe the incidence and mortality have either leveled off or are decreasing (2).

Among females, increasing incidence has been reported from Canada, Italy, Denmark, United States and Australia (3). Mortality rates from this cancer is particularity high among males from Eastern and Southern Europe. South America (Southern Brazil, Uruguay and Argentina) shows the highest mortality rates in males, worldwide (3).

Mortality due to this cancer is very rare among females, accounting for only 0.4% of all deaths due to cancer around the world (4).

In Serbia laringeal cancers account for 3.7% in overall new diagnosed cancers in males and for 0.5% in women. Laringeal cancers account for 0.6% in men and 0.1% in femalesof all deaths by cancer (4).

According to data from developed countries, the prognosis for all patients with laryngeal cancer has remained unchanged since the mid-1970s, with a relative survival rate of 60-65% after five years, for all stages and all forms of treatment (4).

The aim of the paper was to evaluate laringeal cancer trends and five year survival rate among patients in the Nishava District.

## Methods

The data were obtained from the Population Based Cancer Register of Serbia. The study period was 1999 to 2008. The survival was study consisted of all the cases registerd under the clinical di-

agnosis codes 320 to 329 over the period January the first 2004 to 31 th December 2008. According to the International Classification of Diseasess 10th revision (ICD-10).

Crude incidence and mortality rates were calculated per 100.000 inhabitans (estemited Census). Standardization was performed by direct method of standardization, with the World population as the standard (5). Incidence and mortality trends were described in general for male and female population for the entire observed period, based on age-standardized incidence and mortality rates.

The survival patterns were estimated by Kaplan-Meier method and the long rank test was applied to test the differences. The Cox proportional hazards regression model was also applied.

## Results

During the period 1999-2008 a total number of 440 cases of laringeal cancer was registered. A total of 401 (91.1%) were male and 39 (8.2%) cases occurred in female.

### *1. Incidence trend of laringeal cancer in male and female in Nishava District in the period from 1999 to 2008*

According to the data in chart 1 a steady increasing incidence trend was observed in both male and female in the study period.

An increasing incidence trend of laringeal cancer in mele based on the standardized incidence rates was registered. The lowest age-standardized incidence rate in male 7,14/100 000 was in 2000 and the highest one was 15,46/100 000 in 2007.

During the period of observation the incidence trend in males was higher compared with the females (Chart 1).

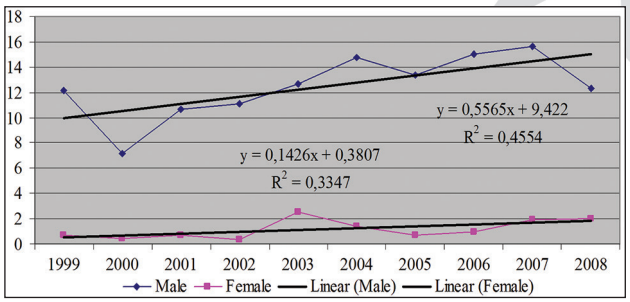


*Chart 1. Incidence trend of laringeal cancer in male and female in the Nishava District in 1999 to 2008*

Value of incidence trend in male: Y= 0,5565x + 9,422 $R^2$= 0,4554

There was an increasing linear incidence trend of laringeal trend of laringeal cancer in female, too.

Value of incidence trend in female: Y=0,1426x + 0,3807, $R^2$=0,3347

The lower standardized incidence rate was 0,38/100 000 (2002) and the highest was 2,53/100 000 in 2003. In this study the overall male to female ratio was 13 : 1.

Mortality trend of laringeal cancer by gender in the period 1999-2008 is in the Chart 2.

### *2. Mortality trend of laringeal cancer in males and females in the Nishava District in the period from 1999 to 2008*

During the entire period of observation a total number of 189 deaths of laringeal cancer were registered, 176 (93.1 %) in male and 13 (6.9%) in female.

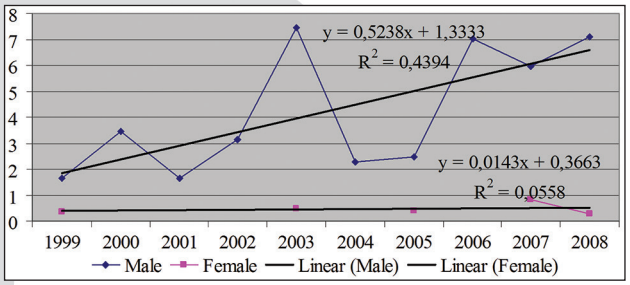


*Chart 2. Mortality trend of laringeal cancer in male and female in the Nishava District in the period from 1999 to 2008*

Value of mortality trend: Y= 0,5238x +1,3333, $R^2$= 0,4394

Value of mortality trend: Y=0,0143x + 0,3663, $R^2$= 0,0558

There was an increasing mortality trend in males and in females mortality trend stagnants. Rapid increase of mortality rates was recorded in men in 2003 and 2006.

Dieing among females of laringeal cancer was not recorded in several years (2001,1003, 2005, 2007).

### *3. Descriptive characteristics of studied patients*

During the period 2004-2008, there were 254 cases of laringeal cancer (230 male and 24 female). The average age of males was 61.7±10.97

(range 34-86) and of females 61.8±12.3 (36-86). 109 patients died.

Among patients who died from the laringeal cancer in the period 2004 to 2008 there were 140 (54.3%) younger than 64 and other 114 (45.7%) were older than 65. In 204 (80.3%) patients the histological finding was squamous cell carcinoma (SCS).

Of the 254 evaluable patients, two thirds had a glottic cancer while approximately one-third of the cancers were supra or subglottic. Descriptive characteristics of the patients are showed in table 1.

***4. Survival rates of laringeal cancer***

The survival rate of patients with the laringeal cancer by months is in the Chart 3.

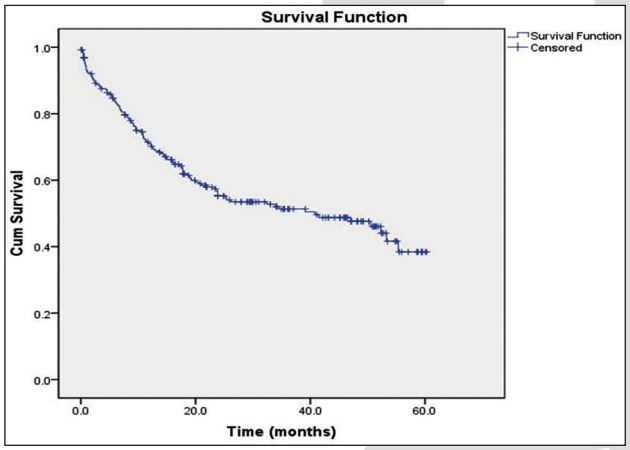


*Chart 3. 5-year survival of laringeal cancer in the Nishava District*

*Estimation is limited to the largest survival time if it is censored.

During the first year after the diagnosis, the survival rate was 70.2%, after second 55.2%, after third 51.3% and after fourth year the survival rate was 47.6%. The overall 5-year survival rate was 38.4%. The mean observed survival was 35.1(95%; 31.876-38.369) months and median was 40.8(95%, 23.054-58.546).

The results of Cox regression analysis are shown in table 2.

***Analisys by gender***

According to the data in table 2, males were died significantly more compared with females. Hazard ratio showed that men had about 1,14 times higher risk of dieing from the laringeal cancer than females.

***Analisys by age***

There wasn't statistically significant between males and females, but hazard ratio showed that patients older than 65 years of age had about 1,025 higher risk of dieing compared with patients who were younger than 65, (table 2).

*Table 1. Descriptive characteristics of patients with laringeal cancer from Nishava District in the period 2004-2008*

| Characteristics | Number/Percent | Average age |
|---|---|---|
| Males | 256 (93%) | 61.7±10.97 (range 34-86) |
| Females | 19 (7%) | 61.8±12.3 (range 36-86) |
| < 65 years of age | 140 (54.3%) | |
| > 65 years of age | 117 (45.7%) | |
| Pathohistological type of cancer | 204 (80.3%) squamous cell carcinoma | |
| Localization | 2/3 of all cancer were glottic and other 1/3 were supra or subglottic cancers | |

*Table 2. Risk factors in Cox regression analysis (SPSS)*

| Variables | B | SE | Wald | df | Sig. | Exp(B) | Lower | Upper |
|---|---|---|---|---|---|---|---|---|
| Gender | 0,131 | 0,369 | 0,126 | 1 | 0,723 | 1,14 | 0,553 | 2,348 |
| Prime localization | -0,147 | 0,247 | 0,356 | 1 | 0,551 | 0,863 | 0,532 | 1,4 |
| Patohyst. type | -0,319 | 0,249 | 1,64 | 1 | 0,2 | 0,727 | 0,446 | 1,185 |
| Age | 0,025 | 0,009 | 8,088 | 1 | 0,004 | 1,025 | 1,008 | 1,043 |

**Discussion**

The data from this paper showed a steady increasing incidence trend of laringeal cancer in both males and females in the Nishava District. A greater increase of incidence was observed in males than in females.

There was an increase mortality trend in males and in females mortality trend stagnates. According to the presented results, laringeal cancer mortality in females in the Nishava District is substantially lower in females than in males. The changes over time in time trends of laringeal cancer between males and females could partly explaned by difference in distribution of the main risk factors, tobacco smocking and alcohol consumption.

The incidence rate was nearly three times higher in the Central Serbia and in the Nishava District compared with the same rate in males and females worldwide. In females the incidence rate was higher about 4,7 times more compared with the ASR (W) in females worldwide.

Laryngeal cancer is generally uncommon in males and very rare in females (2). Laryngeal cancer is the eleventh most common form of cancer among males worldwide (1). In the Central Serbia, laringeal cancer was the sixth most common cancer in males (5).

In view of it's incidence in 2008 laringeal cancer ranked sixth place in males in the Nishava District accounting for 3.7% of all malignant neoplasms. It accounts for 0.6% of all malignant neoplasms in females (5). In Brasil it accounts for 3.8% in males and 0.6% in females of all deaths by cancer (4).

According to the data from Globocan the ASR (W) for males in 2008 was 4.1 and for females 0,6. The same rate among males in the Central Serbia was 13.5 and in the Nishava District the rate was higher, and it′s value was 14.5. In the same year, ASR (W) in females in the Central Serbia was 1.4 and it was slightly lower in females in Nishava District (1.2).

After a steady increase since the 1950s, laryngeal cancer mortality had tended to level off since the early 1980s in men from most European countries (3). Age-standardized mortality rate in men in Central Serbia was 5.9 and in Nishava District the age-standardized mortality rate in males was 5.2. In females age-standardized mortality rate was 0.5, and in Nishava District it was 0.1 (5). Mortality of males from laringeal cancer was 2.2. and mortality of females was 0.3.

According to the presented results the average age of the studied patients was similar males: 61.7±10.97 and females: 61.8±12.3.

The disease is predominantly found in patients aged from 50 to 70 years, although in developing countries many cases are diagnosed in individuals in their fifth decade of life.

The majority of the patients in this study were male (93% vs 7%). Dechaphunkul (2011), also find out that 92.3% of patients were male (1). Raitiola (2000) showed similar data, there were 95% of male and only 5% female.

In this study males suffered from laringeal cancer 13 times more than females. The overall sex ratio was 13: 1. Worlwide, it's values varies beteen 4: 1 to 20: 1 (4) with increasing proportion of female a common finding (7).

According to the case-control study done by Arsenijevic and all. (2010), 91% of the patients with laringeal cancer were males who were 60 years old in average.

In a case-control study conducted in the Metropolitan Region of Sao Paulo, between January 1999 and December 2001, 63% of the laryngeal cancer cases occurred in the age group from 50 to 70 years (4).

The patients from this study was younger in average than patients from other studies. The median age of the patients from Finland was 64 years (9). According to our findings 80.3% of all studied patients the histological finding was SCS and it is less than in the literature. For example, Raitiola (2000) showed that 98% of all patients in their study had SC laringeal cancer (9).

According to data from developed countries, the prognosis for all patients with laryngeal cancer has remained unchanged since the mid-1970s, with a relative survival rate of 60-65% after five years, for all stages and all forms of treatment (6).

According to the presented results there were 54.3% patients younger than 64 who died from laringeal cancer and other 45.7% were older than 65. Piccirilo et al. (2001) found about 66% of the people who had laringeal cancer older than 60 years of age.

In 1994, the majority of laringeal cancer in England and Wales was recorded as occurring in the area of the glottis, followed by the supraglottis, with a small proportion in the subglottis and laryngeal cartilages. A large proportion remained unspecified (11).

In this study, 204 (80.3%) patients the histological finding was SCC and it accounts for more than 90%. Squamous cancer was by far the most common histology (94.1% in Ontario OCR, 94.6% in SEER), (9,10).

The overall improvement in the prognosis was seen in Europe from 1978 to 1989, where the five-year survival increased from 58% to 63% (6-9).

In the period 2003-2007, in the United Kingdom, one-year survival rate for laringeal cancer was 85% and five-year survival rate was 66% (11).

Survival has decreased among patients with laryngeal cancer during the past 2 decades in the United States and during this same period, there has been an increase in the nonsurgical treatment of laryngeal cancer (12)

In a study in India, the five-year relative survival rate was 58.3% for glottic laryngeal cancer cases and 31.4% for supraglottic laryngeal tumor cases (1).

Five-year survival from laringeal cancer is over 60% in both males and females from Europe (13). For patients with glottic carcinoma diagnosed in 1993–1997 the relative five year survival was 87% (14). Five-year survival from glottic laringeal cancer is 81% (9). According to the data of the National Cancer Data Base, based on patients diagnosed from 1998-1999. which were published in the AJCC staging manual the survival rates for glottis are: 90%, 74%, 56%, 44%.

Robbins conducted a study of disease-free survival among males and females with laryngeal cancer and did not find any significant survival differences between men and women with regard to local and regional tumor control (22).

Survival rates are lower in developing countries than in developed countries (4). In a study in India, the five-year relative survival rates were 58.3% for glottic laryngeal cancer cases and 31.4% for supraglottic laryngeal tumor cases. The five-year observed survival was 53.1% for those with localized cancer and 17.8% for patients with regional extension (23).

## Conclusion

An increasing incidence and mortality trend was found among patients with laringeal cancer in population of the Nishava District. The five-year survival rate was 38.4%. Male gender and advaced age were associated with significanty reduced survival. Earlier diagnosis and better adoption of integration therapeutic schemes may also have favorably influenced laryngeal cancer survival rates and consequently mortality trends.

## References


1. *Dechaphunkul T. Epidemiology, Risk Factors, and Overall Survival Rate of Laryngeal Cancer in Songklanagarind HospitalJ Med Assoc Thai 2011; 94 (3): 355-60*

2. *Coleman MP, Estève J, Damiecki P, Arslan A, Renard H. Trends in cancer incidence and mortality. IARC Sci Publ. 1993; (121): 1-806*

3. *Jensen OM, Parkin DM, Lennan R, Muir CS, Skeet RG. Cancer registration. Principles and Methods. Lyon (France): International Agency for Research on Cancer; 1991.*

4. *Filho VW. The epidemiology of laryngeal cancer in Brazil. Sao Paulo Med. J. 2004; 122(5): doi: 10.1590/S1516-31802004000500002: http: //www.scielo.br/scielo*

5. *Incidence and mortality of cancer in Serbia 2010. Institute for Public Health "Dr Milan Jovanovic Batut" Beograd, Serbia, 2010*

6. *Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1990. Int J Cancer. 1999; 80(6): 827-41*

7. *Pisani P, Parkin DM, Bray F, Ferlay J. Estimates of the worldwide mortality from 25 cancers in 1990. Int J Cancer. 1999; 83(1): 18-29*

8. *Piccirillo JF, Costas I. Cancer of the larynx.*

9. *Raiotiola H. Epidemiology, Clinical characteristics and treatment outcome of laringeal cancer. University of Tampere, Medical School of Medicine, Finlad 2000*

10. *Altekruse SF, Kosary CL, Krapcho M, Neyman N, Aminou R, et al. SEER Cancer Statistics Review, 1975-2007, National Cancer Institute. Bethesda, MD, http: //seer.cancer.gov/csr/1975_2007/, based on November 2009 SEER data submission, posted to the SEER web site, 2010*

11. *Coupland HV, Chapman P, Linklater MK, Sehgal A, Møller H, Davies AE. Trends in the epidemiology of larynx and lung cancer in south-east England, 1985–2004. British Journal of Cancer 2009; 100: 167–169*

12. *Hoffman HT, Porter K, Karnell LH, Cooper JS, Weber RS, lenger CS, Ang KK, Gay G, Stewart A, Robinson RA. Laryngeal cancer in the United states changes in demographics, patterns of care, and survival. Laringoscope 2006; 116(Suppl 111): 1-13*

13. *Bosetti C, Talamini R, Levi F, Negri E, Franceschi S, Airoldi L, La Vecchia C. Fried foods: a risk factor for laryngeal cancer? 2002; Br J Cancer 87: 1230–1233*

14. *Kirn-Pompe V. Epidemiological features of laryngeal cancer in Slovenia. ZDRAV VESTN 2002; 71: Suppl. III: 59–63*

15. *Berrino F, Sant M, Verdecchia A, Capocaccia R, Hakulinen T, Esteve J. Survival of cancer patients in Europe. The EUROCARE Study. Lyon: IARC; 1995. (IARC Scientific Publications No. 132) .*

16. *Berrino F, Gatta G. Variation in survival of patients with head and neck cancer in Europe by the site of origin of the tumours. EUROCARE Working Group. Eur J Cancer. 1998; 34 (14 Spec No): 2154-61 .*

17. *Berrino F, Capocaccia R, Esteve J. Survival of cancer patients in Europe. The EUROCARE-II Study.). Lyon: IARC; 1999. (IARC Scientific Publications No. 151)*

18. *Cosseti M,Yu GP, Schatnz SP. Five-year survival rates and time trends of laringeal cancer in the US population. Arch Otolaringol Head and Neck Surg. 2008; 134(4): 370-9*

19. *Arsenijevic S, Pantovic V, Gledovic Z, Stojanovic J, Belic B. Demographic characteristics of patients with laryngeal cancer and their socioeconomic status. J BUON 2010; 1(15):*

20. *Arsenijević SŽ, Belić B, Đonović NŽ. Analiza preživljavanja pacijenata obolelih od karcinoma larinksa i hipofarinksa zavisno od stadijuma bolesti. Medicus 2004; 5 (2): 32-35*

21. *Skarsgard DP, Groome PA, Mackillop WJ, Zhou S, Rothwell D, et al. Cancers of the upper aerodigestive tract in Ontario, Canada, and the United States. Cancer, 2000; 88(7): 1728-38*

22. *Robbins KT. Prognostic and therapeutic implications of gender and menopausal status in laryngeal cancer. J Otolaryngol. 1988; 17(2): 81-5*

23. *Sankaranarayanan R, Ramanakumar AV, Yeole BB. Survival from glottic and supraglottic laryngeal carcinoma in Mumbai (Bombay), India. Oral Oncol. 2003; 39(7): 656-63*

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# Psychological status of premenopausal women before and after hysterectomy

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## Abstract

**Objective:** To investigate the changing trend, degree and impact factors of women's psychological status before and after hysterectomy.

**Methods:** The psychological status of 98 women who received hysterectomy was investigated with prospective study method at the time of admission and 3 months after hysterectomy.

**Results:** The results of psychological self-assessment scores showed that the postoperative scales of BDI, HAMD and SCL-90 (except paranoid factor) were significantly lower than the preoperative ones. The morbidity of moderate and severe depression was decreased from preoperative 48.98 % to 13.54%. The changes of SCL-90 were varied among different age groups. The scales of postoperative BDI and HAMD were significantly positively correlative to preoperative scales; before hysterectomy, HAMD scales were negatively correlated to women's educational level, but positively correlated to chief complaints. However, after hysterectomy, HAMD scales were related positively to parity and medical complications, but negatively related to patients' age.

**Conclusion:** Hysterectomy is conducive to the physical and mental health of patients; perioperative depression often occurs in young and less-educated women with multiple parities and complaining of dysmenorrhea and/ or menorrhagia and other diseases.

**Key words:** Investigation, hysterectomy, psychological status.

## Introduction

Hysterectomy is the gynecological surgery that is most commonly performed. About 600,000 women undergo hysterectomy each year only in the United States. Due to the special social-psychological-sexual roles of uterus, the incidence of mental disorders caused by relevant diseases and surgeries is relatively high. With the changes in social - biomedical model, the research on women's psychological change before and after hysterectomy has become the focus of the psychosomatic medical researches [1-4]. In this paper, a perspective study method was adopted for the analysis of the changing trend, degree and impact factors of the psychological status of women before and after hysterectomy who suffered from pre-menopausal benign diseases, which aims to find out various risk factors in perioperative period with a negative impact on the psychological status of patients and the people susceptible to mental disorders, so as to provide the basis for postoperative rehabilitation and mental health in a targeted way and ensure the perioperative physical and psychological health of patients.

## Materials and methods

### *Objects*

98 unmenopausal women who received hysterectomy for benign diseases from January 2012 to October 2012 in our hospital were investigated, of which the youngest age was 34 years old and the oldest age 52 years old (mean 44.8 years old) and the years of education were from 5 to 15 years (average 8.27 years ). All the patients had no obvious cerebral organic diseases, mental retardation, past history and family history of mental disorders or damage of vital organs, and could understand and independently completed all self-rating scales. They neither had major stressful life experiences in the past year nor receive any surgical treatment within the previous six months.

***Investigation methods***

Psychological test was conducted on the patients at the time of admission and 3 months after hysterectomy respectively in the form of questionnaire survey. In the measurement tools, psychological self-rating scales adopted the Symptom Checklist (SCL-90), Beck Depression Inventory (BDI), Self-Rated Health Measurement Scale and Self-Concept Scale; nurse-administered rating scale selected the Hamilton Depression Rating Scale (HAMD) of the 24 item version. All the patients were evaluated by a professionally trained medical staff.

***General information***

The 98 patients, with the shortest duration of 3 months and the longest duration of 14 years, included 52 cases of chief complaint of menorrhagia, 26 cases of dysmenorrhea, 16 menorrhagia and dysmenorrhea cases and 4 asymptomatic cases. There were 13 patients with previous surgical history; 37 with mild medical complications; 72 undergoing total hysterectomy, 26 subtotal hysterectomy, 18 unilateral salpingo oophorectomy and 11 bilateral salpingo oophorectomy.

***Evaluation standard of depressive state***

Beck Depression Inventory (BDI) determined the extent of depressive state according to the BDI score: $\leq 4$ points for no depression or imperceptible depression, 5 to 13 for mild depression, 14 to 20 for moderate depression, $\geq 21$ for severe depression [5]. The Hamilton Depression Scale evaluates the degree of depression according to the HAMD score: $\leq 8$ points for no depression, 9 to 20 for mild depression, 21 to 35 for moderate depression, $> 35$ for severe depression, in which a patient whose HAMD scored more than 20 points could be diagnosed as depression [6].

***Using Rules for Self-Rated Health Measurement Scale (SRHMS) and Self-Concept Scale***

In SRHMS, self-rated measurement was conducted by Xu Jun, etc. from such three aspects as physiology, psychology and society (3 subscales) and nine dimensions according to the change from the biomedical model to modern medical model. It is an evaluation of health status suitable for people over the age of 14. The theoretical maximum values of the three subscale scores of physical, mental and social health and the total score of SRHMS were 170,150,120 and 440 respectively. The test-retest reliability of SRHMS was 0.857, with a relatively high content validity. The higher SRHMS scores, the better the health status was. Self-Concept Scale was prepared in 1965 by Williams, a psychologist in the Department of Mental Health, Tennessee, U.S.A. and was supplemented and revised by the American scholar Petersen later [7]. The scale had 10 self rating scores, which, in addition to total self rating score and self-criticism, was also divided into two dimensions: structural dimension (self-identity, self-satisfaction, self-action) and content dimension (physical self, moral and ethical self, psychological self, family self and social self). The domestic application of this scale shows a high reliability and validity.

***Statistical analysis***

All the data were expressed as ±s. The groups were compared by the t test. The correlations between HAMD score and influence factors were subjected to multiple regression analysis.

**Results**

The patients were divided into four age groups, i.e. <40 years old, 40 to 45 years old, 45 to 50 years old and > 50 years old. The preoperative numbers of patients in each group were respectively 14, 36, 21 and 12, and 14, 34, 19, 12 postoperatively. There were 2 patients loss to follow-up, with the dropout rate of 2.04%.

***Depressive state tendency before and after operation***

The BDI and HAMD scores before and after operation are shown in Table 1. The results showed that BDI and HAMD scores in the four age groups decreased significantly after operation compared with those before operation, with a significant difference ($P < 0.05$).

***Depressive state extent before and after operation***

Self-rating and nurse-administered rating methods were respectively used to investigate the degree of depression in the patients before and after op-

*Table 1. BDI and HAMD scores before and after operation*

| Age | BDI score | | HAMD score | |
|---|---|---|---|---|
| | Before | After | Before | After |
| < 40 | 13.92±5.58 | 10.27±3.34* | 15.32±4.15 | 11.85±4.56* |
| 40～< 45 | 12.16±4.28 | 9.01±4.08* | 14.35±5.01 | 10.64±4.61* |
| 45～<50 | 11.25±4.36 | 8.24±4.25* | 14.92±4.15 | 10.57±4.81* |
| >50 | 12.45±6.41 | 8.74±5.04* | 13.82±3.89 | 9.24±5.24* |
| Total | 12.63±4.92 | 9.16±4.51* | 14.62±3.28 | 10.59±4.68* |

**Compared to the results before operation, P<0.05*

*Table 2. BDI scores before and after operation*

| BDI score | Before | | After | |
|---|---|---|---|---|
| | Case No. | Percentage | Case No. | Percentage |
| ≤4 | 6 | 6.12% | 43 | 44.78% |
| 5～13 | 37 | 37.76% | 45 | 46.88% |
| 14～20 | 46 | 46.94% | 8 | 8.34% |
| ≥21 | 9 | 9.18% | 0 | 0 |

*Table 3. HAMD scores before and after operation*

| HAMD value | Before | | After | |
|---|---|---|---|---|
| | Case No. | Percentage | Case No. | Percentage |
| ≤8 | 11 | 11.22% | 34 | 35.42% |
| 9～20 | 39 | 39.80% | 49 | 51.04% |
| 21～35 | 42 | 42.86% | 12 | 12.50% |
| >35 | 6 | 6.12% | 1 | 1.04% |

eration. The changes of self-rating depression status before and after operation are shown in Table 2 and the results of nurse-administered rating before and after operation are listed in Table 3.

According to the criteria of BDI scores, the results in Table 2 showed that the postoperative depression status greatly improved compared with the preoperative one. There were 6 patients belonging to no depression or imperceptible depression before operation, but the number rose to 43 postoperatively, 37 patients with preoperative mild depression, which increased to 45 postoperatively, 55 patients with preoperative moderate and severe depression which dropped to 8 postoperatively. In self-rating, the incidence rate of moderate and severe depression decreased from 56.12% before operation to 8.34% after operation.

According to the HAMD rating rule, the results in Table 3 showed that there were 11 without preoperative depression, which increased to 34 postoperatively, 39 with preoperative mild depression which rose to 49 and 48 with preoperative moderate and severe depression which dropped to only 13 postoperatively. According to the diagnostic criteria, when a patient's HAMD score is more than 20, he/she can be diagnosed as depression. The results of Table 3 showed that the incidence of depression in this group was 48.98% before operation which decreased to 13.54% after operation. The postoperative incidence of depression decreased significantly compared with the preoperative one.

The statistics showed that the consistency between the BDI score and HAMD score was 0.92, having a good coincidence rate.

***Factors affecting depressive state***

The scales of postoperative BDI and HAMD were highly significantly correlative to preoperative scales (r = 0 9135, P <0. 01; r = 0. 9254, P <0. 01). In order to understand the degree of influence of a variety of social factors on HAMD score of the patients before and after operation, multiple regression analysis was made between HAMD scores and age, incidence years, years of education, occupation, times of gestation and parity, history of previous surgery, existing number of

children, whether there are medical complications or not and the extent of surgery. The results suggested that the preoperative HAMD (Y) score was negatively correlative to years of education (X1), and positively correlative to main symptoms (X2), and had no significant relationship with other factors. The regression equation was expressed as (Y = - 5014 X1 + 0. 3285X2) (P <0.05); the postoperative HAMD score (Y) was positively correlated to parity (X1) and medical complications (X 2), negatively correlated with age ( X 3) and had no significant relationship with other factors. The regression equation was expressed as (Y = 0. 3648X + 0.2981X 2 - 0. 3014X 3) (P <0 05).

### *SCL-90 survey results*

The SCL-90 survey results and comparison between before and after hysterectomy are shown in Table 4. The comparison of each factor score of SCL-90 before and after hysterectomy showed that on the whole, the postoperative positive number of items and somatization factor score were significantly lower than the preoperative ones, and the scores of compulsion, depression, anxiety, hostility, terror, interpersonal sensitivity and psychotic factor apparently decreased compared with preoperative relevant scores, showing a significant difference (P <0.01). The degree of change in patients varied in different ages, in which the two groups aged from 40 to 45 and 45 to 50 showed an obvious changing trend, while although the scores of the other two groups had a general decline compared with the preoperative scores, there were few items with significant differences.

### *SRHMS scores of different age groups*

There were no significant differences between the SRHMS scores of different age groups (P> 0.05), but the physical and overall health levels gradually declined with increasing age, and the mental health and social health status presented a V0 trend with lower middle part and higher both sides. We compared the SRHMS rating results of the 98 patients (study group) with those of patients living in economically developed areas (control group). The physical health of the former was better than the latter, while social health score was lower (Table 5).

*Table 4. SCL-90 survey results and comparison between before and after hysterectomy*

| Item | <40 | | 40～<45 | | 45～<50 | | >50 | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After | Before | After | Before | After |
| Total score | 32.15±9.42 | 27.31±8.49 | 48.54±7.56 | 29.37±8.47** | 44.58±9.18** | 29.45±9.46 | 30.14±8.14 | 19.48±8.99* | 41.25±9.77 | 27.16±8.81** |
| Positive list | 23.47±7.52 | 18.64±8.48 | 30.25±9.96 | 16.72±8.76** | 27.41±9.54** | 19.48±9.98 | 21.58±8.45 | 19.87±9.82 | 26.41±9.12 | 17.45±7.49** |
| Somatic | 0.41±0.28 | 0.40±0.34 | 0.74±0.36 | 0.35±0.32** | 0.82±0.53 | 0.49±0.35* | 0.46±0.38 | 0.38±0.34 | 0.61±0.58 | 0.43±0.38** |
| Compulsory | 0.39±0.25 | 0.34±0.30 | 0.72±0.42 | 0.34±0.31** | 0.62±0.36 | 0.59±0.45 | 0.51±0.31 | 0.48±0.34 | 0.68±0.36 | 0.34±0.25** |
| Sensitivity | 0.37±0.23 | 0.36±0.32 | 0.64±0.52 | 0.29±0.24** | 0.26±0.24 | 0.24±0.21 | 0.42±0.31 | 0.28±0.21* | 0.64±0.25 | 0.29±0.36** |
| Depression | 0.47±0.32 | 0.42±0.35 | 0.68±0.59 | 0.24±0.24** | 0.34±0.30 | 0.32±0.31 | 0.38±0.32 | 0.24±0.21* | 0.58±0.35 | 0.24±0.23** |
| Anxiety | 0.34±0.28 | 0.34±0.24 | 0.51±0.36 | 0.31±0.36* | 0.51±0.36 | 0.34±0.31** | 0.45±0.37 | 0.38±0.34 | 0.47±0.36 | 0.24±0.15** |
| Hostility | 0.27±0.19 | 0.24±0.20 | 0.49±0.36 | 0.30±0.29* | 0.41±0.36 | 0.29±0.28* | 0.34±0.21 | 0.31±0.25 | 0.61±0.36 | 0.34±0.31** |
| Panic | 0.43±0.34 | 0.39±0.37 | 0.40±0.25 | 0.29±0.28* | 0.34±0.32 | 0.20±0.18* | 0.29±0.21 | 0.24±0.21 | 0.58±0.32 | 0.36±0.21** |
| Paranoid | 0.45±0.23 | 0.43±0.38 | 0.25±0.22 | 0.21±0.19 | 0.24±0.21 | 0.21±0.19 | 0.18±0.16 | 0.14±0.12 | 0.24±0.21 | 0.22±0.19 |
| Neurotic | 0.39±0.27 | 0.37±0.34 | 0.31±0.25 | 0.11±0.08** | 0.31±0.28 | 0.24±0.21 | 0.24±0.21 | 0.22±0.19 | 0.54±0.36 | 0.34±0.32** |
| Others | 0.41±0.34 | 0.44±0.32 | 0.61±0.45 | 0.20±0.18** | 0.52±0.33 | 0.31±0.26* | 0.52±0.41 | 0.48±0.37 | 0.61±0.44 | 0.39±0.34** |

**P<0.05, **P<0.01, compared to the results before operation*

*Table 5. SRHMS scores of different age groups*

| Age | < 40 | 40～< 45 | 45～<50 | >50 |
|---|---|---|---|---|
| Physical health | 140.31±34.12 | 142.36±48.69 | 141.37±42.36 | 141.98±54.85 |
| Mental health | 113.58±24.69 | 105.36±65.35 | 106.34±42.65 | 116.34±54.25 |
| Social health | 89.24±24.66 | 82.34±31.26 | 81.91±24.69 | 88.15±28.49 |
| Total score | 334.91±84.25 | 339.24±139.14 | 334.85±106.59 | 348.51±134.69 |

*Table 6. Correlation between self-concept and SCL-90 and SRMHS scores*

| Self-concept | SCL- 90 total score | SRHMS | | | |
|---|---|---|---|---|---|
| | | Physical health | Mental health | Social health | Total score |
| Self-identification | -0.42** | 0.31** | 0.38** | 0.35** | 0.41** |
| Self-satisfaction | -0.36** | 0.21** | 0.34* | 0.36* | 0.36** |
| Self-action | -0.48** | 0.26* | 0.35* | 0.28** | 0.28* |
| Physical self | -0.46** | 0.37** | 0.26** | 0.42** | 0.34** |
| Ethical self | -0.42** | 0.18** | 0.37** | 0.40* | 0.34** |
| Mental self | -0.38** | 0.25* | 0.29* | 0.29* | 0.29** |
| Family self | -0.41** | 0.22** | 0.41** | 0.34** | 0.28** |
| Social self | -0.46** | 0.30** | 0.36** | 0.37* | 0.41* |
| Total score | -0.45** | 0.24* | 0.33* | 0.35** | 0.30** |
| Self-criticism | 0.06 | 0.03 | 0.01 | -0.06 | -0.02 |

**P<0.05; **P<0.01*

***Correlation between self-concept and SCL-90 and SRHMS scores***

Except for a reverse factor (self-criticism), the scores of the remaining eight factors and their total score were significantly negatively correlated with SCL-90 total score and the scores of all factors (P <0 01), and were significantly positively correlated with SRHMS total score, subscale scores and the scores of all factors (P<0. 05, P<0. 01). The correlations are shown in Table 6.

***Regression analysis of metal health factors***

In order to further study the relationship between mental health and self concept, stepwise regression analysis was conducted according to A = 0.05 with SCL-90 total score and mental health score in SRHMS as dependent variables and self-concept factors as independent variables. The results showed that self identity, physical self and mental self entered the SCL-90 regression equation (B = 0.24, 0. 28, 0.22); self identity, social self, physical self and ethical self were put into the SRHMS regression equation (B = 0. 34, 0.28, 0.22, - 0.23). Self identity and physical self came into the equation in regression analysis, indicating that these two factors exert a relatively great influence on psychology of patients.

**Discussion**

Many problems in the field of gynecology and obstetrics involve psychosomatic medicine. Epidemiological surveys have showed that, when nearly half of premenopausal women are disturbed by gynecological symptoms such as menorrhagia and/or dysmenorrhea, and premenstrual tension, psychosomatic influences of diseases on patients make them nervous, down in spirits, agitated and painful and thus produce multiple psychiatric symptoms such as irritability, crying, anxiety and depression, which significantly influences the life quality and status of patients and even causes degradation of various social functions [8-11]. Hysterectomy is a major means for curing these diseases. With the development of psychosomatic medicine, people have started to pay attention to the change of psychological status of postoperative patients losing reproductive organs. In earliest researches, Clayton found through the survey on postoperative patients that over half of patients suffered from such symptoms as lack of power, insomnia, depression and sexual dysfunction after operation. He thought that these symptoms were caused by hysterectomy and called as postoperative syndrome of hysterectomy [12]. However, a lot of subsequent prospective

studies denied this viewpoint and found that operation could recover patients' daily activities, social contact and sexual life which were reduced due to disease before operation and was conducive to psychosomatic health of patients while removing the disease [13,14]. In this study, the results of depression evaluation before and after operation showed that, no matter in self rating or nurse-administered rating, the scores of patients after operation decreased significantly compared with those before operation, the depression of most patients disappeared or significantly improved after operation, and the morbidity of depression was significantly lower than that before operation and the morbidity of moderate and severe depression decreased from 48.98% before operation to 13.54% after operation. The number of positive items and somatization factor score in SCL-90 after operation significantly decreased, with a significant difference compared with those before operation and the total score, compulsion, depression, anxiety, hostility and interpersonal sensitivity factor scores significantly decreased, with a significant difference compared with those before operation. This indicates that diseases affect the mental health of patients and cause severe depressive tendency and various psychiatric symptoms while affecting their physical health and that operation releases factors influencing the mental health of patients while curing physical diseases, thus alleviating the depressive state and improving the psychological status. Hysterectomy is conducive to both mental and physical health of patients.

Psychological self and interpersonal relationship in SCL-90 had a significant negative correlation, indicating that good interpersonal relationship has an important influence on the mental health of patients. Females are sensitive to the change of interpersonal relationship. Females also have specific characteristics in physiology. Endocrine changes easily cause mood fluctuation and thus reduce emotive behavior and adjustment ability. In addition, due to the influence of customs and traditional habits, females undertake more responsibilities of taking care of the family. Plus the attack of disease and work pressure, once changes of body, emotion, attitude and behavior occur, they are easily sensitive to interpersonal relationship. Therefore, various interpersonal conflicts might occur, resulting in mental imbalance. It can be seen from relevant analyses that the improvement of interpersonal relationship is an effective method for improving the psychological self concept of patients [15-17]. Therefore, the healthy development of patients is of great significance to preventing interpersonal relation disturbance and improving the interpersonal relationship with people around.

In this study, depression scores of patients both in self rating and nurse-administered rating were positively correlated to the levels before operation; HAMD score before operation was inversely proportional to the years of education but in direct proportion to major symptoms; HAMD score after operation was positively correlated to parity and underlying medical disorders but negatively correlated to age. These results indicate that, in this study, the postoperative depressive status of patients is affected by the preoperative level and that susceptible population of moderate and severe depression before and after operation are mainly young and less-educated women with multiple parities and complaining of dysmenorrhea and medical complications [18]. It means that the preoperative evaluation of psychological status is valuable for predicting the change of postoperative psychological status and the result of self psychological rating is highly consistent with that of nurse-administered rating. Therefore, medical workers can make use of self psychological rating information of patients to provide psychological counseling and intervention therapy for high risk population susceptible to dysphrenia before and after operation as early as possible so as to reduce the occurrence of mental disorders in perioperative period.

In this study, various factor scores in SCL-90 of patients in different age groups had different degrees of change before and after operation. The change of patients between 40 and 50 before and after operation was basically the same with the general level; SCL-90 factor scores of patients below 40 and above 50 after operation generally decreased compared with those before operation, but there were few items with significant differences, indicating that the mental health level of females in these two age groups was lower than that of patients between 40 and 50 possibly due to: special social-psychological-sexual roles of uterus. For young women with a low education level, uterus not only undertakes the social task of breeding off-

spring, but also is the symbol of their female identity and sexual attraction. When patients are forced to accept the operation due to a disease, they feel depressed about the incurred loss of reproductive function and sexual organs, thus resulting in poor mental adaptation after operation. Women between 40 and 50 shoulder more social and family responsibilities, while diseases may aggravate patients' mental burden. Operation helps patients get rid of sufferings from diseases and also feel more energetic to accomplish various social tasks. Therefore, the psychological status of patients improves significantly after operation. Patients above 50 suffer from various medical complications such as coronary disease, hypertension and diabetes mellitus etc. besides gynecological diseases, which are mostly accompanied by mental disorder. Hysterectomy cannot improve these complications or alleviate mental disorder caused by these diseases [19-21]. Therefore, some factor scores in the psychological evaluation of patients above 50 did not change greatly before and after operation.

In regression analysis, self-concepts having great influence on the total score of SCL-90 include self identity, physical self and psychological self; self concepts having great influence on mental health in SRHMS include self identity, social self, physical self and moral-ethical self. It can be seen that self identity and physical self are common factors influencing the mental health of patients. This reflects that the correct understanding of body, health status, appearance and self status is an important measure for preventing mental diseases.

This study found some susceptible factors influencing the psychological status of patients in perioperative period before and after hysterectomy, especially high risk groups of depression in perioperative period. It is expected that clinical workers can pay attention to the mental health of patients and ensure the physical and psychological healthy of each patient in perioperative period while curing his/her physical diseases.

## References


1. *Ammar AS, Mahmoud KM, et al. Effect of adding dexamethasone to bupivacaine on transversus abdominis plane block for abdominal hysterectomy: A prospective randomized controlled trial. Saudi J Anaesth, 2012; 6(3): 229-33.*

2. *Solomon ER, Muffly TM, et al. Common postoperative pulmonary complications after hysterectomy for benign indications. Am J Obstet Gynecol, 2012; 12(38): 489-93.*

3. *Blikkendaal MD, Twijnstra AR, et al. Vaginal cuff dehiscence in laparoscopic hysterectomy: influence of various suturing methods of the vaginal vault. Gynecol Surg, 2012; 9(4): 393-400.*

4. *Naphatthalung W, Cheewadhanaraks S. Prevalence of endometriosis among patients with adenomyosis and/or myoma uteri scheduled for a hysterectomy. J Med Assoc Thai, 2012; 95(9): 1136-40.*

5. *Brouwer D, Meijer RR. On the Factor Structure of the Beck Depression Inventory-II: G Is the Key. Psychol Assess, 2012; 7(16): 334-38.*

6. *Zimmerman M, Martinez J. Symptom differences between depressed outpatients who are in remission according to the Hamilton Depression Rating Scale who do and do not consider themselves to be in remission. 2012; 142(3): 77-81.*

7. *Petersen HV, Domanska K, et al. Validation of a self-concept scale for Lynch syndrome in different nationalities. J Genet Couns, 2011; 20(3): 308-13.*

8. *Oppermann K, Fuchs SC. Physical, psychological, and menopause-related symptoms and minor psychiatric disorders in a community-based sample of Brazilian premenopausal, perimenopausal, and postmenopausal women. Menopause, 2012; 19(3): 355-60.*

9. *Sen C, Morimoto Y, et al. Soy foods and urinary isoprostanes: results from a randomized study in premenopausal women. Food Funct, 2012; 3(5): 517-21.*

10. *Rzymski P, Wysocki PJ, et al. Correlation between insulin resistance and breast elasticity heterogeneity measured by shear wave elastography in premenopausal women - a pilot study. Arch Med Sci, 2011; 7(6): 1017-22.*

11. *Cizza G, Mistry S, et al. Do premenopausal women with major depression have low bone mineral density? A 36-month prospective study. PLoS One, 2012; 7(7): 334-38.*

12. *Clayton AH, Maserejian NN, et al. Depression in premenopausal women with HSDD: baseline findings from the HSDD Registry for Women. Psychosom Med, 2012; 74(3): 305-11.*

13. *Aydin H, Mutlu N. Treatment of a major depression episode suppresses markers of bone turnover in premenopausal women. J Psychiatr Res, 2011; 45(10): 1316-20.*

14. *Cizza G, Nguyen VT, et al. Low 24-hour adiponectin and high nocturnal leptin concentrations in a case-control study of community-dwelling premenopausal women with major depressive disorder: the Premenopausal, Osteopenia/Osteoporosis, Women, Alendronate, Depression (POWER) study. J Clin Psychiatry, 2010; 71(8): 1079-87.*

15. *Anestis MD, Joiner TE. Examining the role of emotion in suicidality: negative urgency as an amplifier of the relationship between components of the interpersonal-psychological theory of suicidal behavior and lifetime number of suicide attempts. J Affect Disord, 2011; 129(3): 261-9.*

16. *Balaguer I, Gonzalez L, et al. Coaches' interpersonal style, basic psychological needs and the well- and ill-being of young soccer players: A longitudinal analysis. J Sports Sci, 2012; 30(15): 1619-29.*

17. *Bay EH, Blow AJ, et al. Interpersonal relatedness and psychological functioning following traumatic brain injury: implications for marital and family therapists. J Marital Fam Ther, 2012; 38(3): 556-67.*

18. *Ramos Olazagasti MA, Shrout PE, et al. The longitudinal relationship between parental reports of asthma and anxiety and depression symptoms among two groups of Puerto Rican youth. J Psychosom Res, 2012; 73(4): 283-8.*

19. *Aisenberg Romano G, Ravid H, et al. The psychological profile and affective response of women diagnosed with unexplained infertility undergoing in vitro fertilization. Arch Womens Ment Health, 2012; 15(6): 403-11.*

20. *Kocelak P, Chudek J. Psychological disturbances and quality of life in obese and infertile women and men. Int J Endocrinol, 2012; 3(18): 445-48.*

21. *Gausia K, Ryder D. Obstetric complications and psychological well-being: experiences of Bangladeshi women during pregnancy and childbirth. J Health Popul Nutr, 2012; 30(2): 172-80.*

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# The Turkish version of self-care of heart failure index, v6

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**Abstract**

**Objective:** The aim of the study was to examine the validity and reliability of Self-Care of Heart Failure Index Scale, v6 (SCHFI, v6) for the Turkish Society.

**Methods:** This is a methodological study whose sample was composed of 170 heart failure patients.

**Results:** In the confirmatory factor analysis, factor loads for maintenance scale was found between .36 and .63, for management scale was found between .38 and .63 and for confidence scale was found between .61 and .78. Item-total score correlations were positive at the statistically significant level between .47 and .69 in the maintenance scale, between .50 and .71 in the management scale and between .71 and .83 in the confidence scale. The Cronbach's alpha coefficient was .79 for self-care maintenance, .63 for self-care management, and .85 for self-care confidence.

**Conclusion:** The Turkish version of "SCHFI, v6" scale is a valid and reliable tool for Turkısh population.

**Practice implication:** The Turkish version of "SCHFI, can be used in the nursing practices and researches.

**Key words:** Heart failure, nursing, reliability and validity, scale, self-care.

## Introduction

Heart failure (HF) is a frequent health problem whose incidence rate increases each year with high mortality and morbidity rates[1]. Heart failure leads to a decline in the quality of life and frequent hospitalization[2,3]. Therefore, self-care behaviors need to be developed in order to enhance positive health behaviors and prevent frequent hospitalization[4]. Compliance to treatment, diet, exercises, weight follow, identifying and managing the symptoms are self-care behaviors associated with heart failure. However, patients fail to perform their self-care due to various reasons[5].

In their study where they examined the heart failure self-care management, Dickson et al. (2008) observed that self-care skills of individuals with heart failure were not sufficient, 61 % of the patients could maintain their self-care while 44 % could manage their self-care properly[6]. Riegel and Carlson (2002) reported in their study that patients suffering from heart failure had difficulty in realizing the changing symptoms and indicators[7]. In the study, patients expressed that they did not perceive sudden weight gain, edema in the ankle, fatigue and difficulty in breathing as indicators of the heart failure. Artinian et al. (2002) reported that the least frequent self-care behaviors were related to the symptom monitoring and management. For instance, patients did not apply to a doctor when extreme fatigue, nausea and weight gain were observed in terms of symptom management and they did not monitor their weights daily and their fluid intake in terms of symptom monitoring[4].

With appropriate self-care practices, health problems can be detected early, protection can be provided and care expenses can be reduced[3]. Thus, principally self-care requirements of the patients should be evaluated. Moreover, effects of nursing interventions that would be applied to the patients need to be assessed. Thus, it is compulsory to measure the self-care behaviors of heart failure patients through appropriate measurement tools but there is not any reliable and valid measurement tool in Turkey. Self-Care of Heart Failure Index Scale is one of the most frequently used scales in the literature. However, a validated Turkish version had not been available.

Firstly, it should be adapted to the Turkish society to be able use it in our country. Even though some concepts are common phenomena for many societies, tests developed in a specific culture and a specific language reflects comprehension, conceptualization and sampling characteristics of that

specific culture. Tests should be examined in detail systematically[8,9,10].

This study was carried out to examine the validity and reliability of Self-Care of Heart Failure Index Scale, v6 (SCHFI, v6) developed by Riegel et al. (2009) for the Turkish society[11].

## Methods

### *Design and sample*

This was a methodological research. The study was conducted in Cardiology outpatient clinics of two university hospitals. It is recommended that sample number should be five or ten times more than the scale item number to perform the factor analysis for scale validity and reliability studies[12]. In this study, it was aimed to reach at least 110 patients with heart failure on the basis of scale item number and 170 patients who were being monitored in the Cardiology outpatient clinics of two university hospitals due to heart failure were included in the research sample. For test-retest purposes, the instrument should be administered for the second time. For test-retest analysis a group of at least 30 is recommended [13,14]. In this study, ten days following the first administration, SCHFIv6 was given to 31 patients who were willing to take part in the retest. Patient, who voluntarily accepted to participate in the research, were literate in Turkish, had no hearing and speaking impairment, had orientation to place-time-person. Age average of the patients was 61.32 (SD 13.28), 53.9 % of them were female and 80.6 % of them were married.

### *Data collection*

Data were collected using the Self-Care of Heart Failure Index, V6 (SCHFI-V6).

### *Instruments*

Self-care of heart failure index, $v_6$ (SCHFI-V6)

Self-Care of Heart Failure Index Scale, version 6 (SCFHI, v6) which Riegel et al. developed in 2004 and revised in 2009 was used in the research to examine the reliability and validity of this scale for the Turkish Society[15,11].

This scale composed of 22 items in total had three scales including self-care maintenance (10 items), self-care management (6 items) and self-care confidence (6 items). Score of each scale is standardized between 0 and 100. In the maintenance scale, one item (8) is reversed and its scoring is made as follows: (sum of section A items -10) * 3.333. Score the management scale only if the patient reported having trouble breathing or ankle swelling in the past interval. Otherwise, even though the patient has answered the questions, answers are ignored. First item (In the past month, have you had trouble breathing or ankle swelling?) is only intended for this purpose and it is not included in the scoring. Scoring of the management scale is made as follows: (sum of section B items – 4)*5. As to the scoring of the self-care confidence scale, it is calculated through the following formula: (sum of section C items – 6) * 5.56. A score of 70 or above obtained on a scale is considered adequate self-care.

In the self-care of heart failure index-v6, internal consistency reliability of self-care maintenance scale (Cronbach alpha) was .55, that of self-care management scale was .59 while that of self-care confidence scale was .82[11].

### *Procedures*

Language validity of the scale was analyzed as the first step of the research conducted to test the validity of the scale for the Turkish society. Inventory which was translated into Turkish by all researchers and a linguist. The researchers reviewed this preliminary Turkish version of the scale and then drafted one Turkish version of the SCHFI. The forward-translated version was then back-translated by a professional bilingual translator unfamiliar with either the English or the Turkish version of the SCHFI to ensure the accuracy of the translation. Translated English form and the original form were compared by the researchers. If the items or response choices of between the forward translated and back-translated instruments did not agree, the choice of words were discussed among the translators until a final version was reconciled[10,14].

Translated Turkish version was submitted to the expert opinion (four faculty members from Faculty of Nursing, two clinical nurses, two doctors from the Department of Cardiology) for an analysis of its content validity. Experts were asked to rate each item on the Turkish version of the SCHFI based on relevance, clarity and simplicity as one (not appropriate at all), 10 (completely appropriate)[9]. Acquiring the final form with expert

opinions, the scale was used in pre-interviews conducted with nine patients.

***Data analysis***

Analysis was conducted using descriptive statistics and appropriate reliability and validity statistical tests using the Statistical Package for the Social Services SPSS 15.0 (SPSS Inc. Chicago IL). Expert opinions for the content validity of the scale were evaluated through Kendall W analysis. Confirmatory factor analysis was used for construct validity[16-18]. Pearson's Product-Moment Correlation Coefficient was used in the reliability analysis as well as item total score correlation and Cronbach alpha analysis. Test-retest measurement was assessed using Pearson Correlation and a dependent t-test with ten days interval[9,10].

***Ethical considerations***

Written permission of Barbara Riegel who developed this scale was received through e-mail to examine reliability and validity of Self-Care of Heart Failure Index Scale in Turkey. Written permissions of Dokuz Eylül University Ethical Committee and the other institutions were also taken. Objective of the research was explained to the participants and written permissions were received from those accepting to participate in the research.

## Results

***Descriptive statistics of the SCHFI***

Descriptive data for each item of SCHFI are shown in Table 1. The minimum and maximum scores for each item of the scale were 0 and 4, respectively. The mean value of the items ranged from 1.24 ± .52 to 3.34 ± .76 (Table 1). The mean score of the patients was 42.76 (SD 15.74) in the maintenance scale, 30.87 (SD 14.77) in the management scale and 36.14 (SD 18.79) in the self-care confidence scale (Table 2).

*Table 1. Descriptive statistics of SCHFI, $V_6$ and item-total score correlations (n= 170)*

| Items | M | SD | r | p |
|---|---|---|---|---|
| **Self-Care Maintenance** | | | | |
| 1. Weigh yourself | 2.24 | .82 | .63 | .001 |
| 2. Check your ankles for swelling | 2.24 | .72 | .59 | .001 |
| 3. Try to avoid getting sick (e.g., flu shot, avoid ill people) | 2.86 | .61 | .58 | .001 |
| 4. Do some physical activity | 1.75 | .78 | .65 | .001 |
| 5. Keep doctor or nurse appointments | 2.77 | .68 | .62 | .001 |
| 6. Eat a low salt diet | 2.31 | .88 | .62 | .001 |
| 7. Exercise for 30 minutes | 1.54 | .82 | .65 | .001 |
| 8. Forget to take one of your medicines | 3.34 | .76 | .49 | .001 |
| 9. Ask for low salt items when eating out or visiting others | 1.88 | .75 | .69 | .001 |
| 10. Use a system (pill box, reminders) to help you remember your medicines | 1.91 | 1.14 | .47 | .001 |
| **Self-Care Management (n: 155)** | | | | |
| 11. How quickly did you recognize it as a symptom of heart failure? | 1.61 | 1.01 | .60 | .001 |
| 12. Reduce the salt in your diet | 2.12 | .91 | .71 | .001 |
| 13. Reduce your fluid intake | 1.44 | .60 | .50 | .001 |
| 14. Take an extra water pill | 1.24 | .52 | .57 | .001 |
| 15. Call your doctor or nurse for guidance | 2.43 | 1.04 | .65 | .001 |
| 16. How sure were you that the remedy helped or did not help? | 1.33 | .78 | .54 | .001 |
| **Self-Care Confidence** | | | | |
| 17. Keep yourself free of heart failure symptoms | 1.67 | .64 | .71 | .001 |
| 18. Follow the treatment advice you have been given | 2.62 | .85 | .75 | .001 |
| 19. Evaluate the importance of your symptoms | 2.14 | .73 | .83 | .001 |
| 20. Recognize changes in your health if they occur | 2.34 | .71 | .79 | .001 |
| 21. Do something that will relieve your symptoms | 1.77 | .75 | .75 | .001 |
| 22. Evaluate how well a remedy works | 1.96 | .76 | .76 | .001 |

*Table 2. Patients' Self-Care of Heart Failure Index, $V_{6\ scores}$*

| SCHFI Scales* | n | Minimum | Maximum | Mean (SD) |
|---|---|---|---|---|
| Self-Care Maintenance | 170 | 6.67 | 96.67 | 42.76 (15.74) |
| Self-Care Management | 155 | 5.00 | 75.00 | 30.87 (14.77) |
| Self-Care Confidence | 170 | .00 | 100.00 | 36.14 (18.79) |

**Score of each scale is between 0 and 100 point.*

*Table 3. Comparison of SCHFI, $V_6$ test-retest score means and correlations*

| SCHFI Scales | n | First Administration Mean (SD) | Second Administration Mean (SD) | t* | p | r | p |
|---|---|---|---|---|---|---|---|
| Self-Care Maintenance | 31 | 50.22 (20.62) | 50.22 (19.59) | .000 | 1.000 | .95 | .001 |
| Self-Care Management | 30 | 30.33 (12.24) | 31.17 (12.50) | 1.000 | .326 | .93 | .001 |
| Self-Care Confidence | 31 | 39.43 (24.40) | 39.96 (18.05) | .251 | .804 | .88 | .001 |

** t- test in dependent groups: degree of freedom, respectively= 30/29/30.*

### *Validity analysis*

### *SCHFI's concordance validity*

Scores of the eight experts were evaluated using the Kendall *W* analysis and no statistically significant difference was found between the scores (for SCHFI, Kendal *W*= .131, p= .576). Consequently, it was determined that expert scores were consistent with one another.

### *Construct validity*

Confirmatory factor analysis was performed to confirm the consistency of scales for construct validity in the study to adapt the Self-Care of Heart Failure Index into Turkish. It was observed in the first confirmatory factor analysis that consistency values were not at the desired levels. However, it was realized that consistency values elevated at the end of confirmatory factor analysis that was carried out after modifications were performed between error variances of 10th and 15th items and 18th and 22nd items in accordance with modification suggestions in the statistical package. Consistency values were determined as follows: "chi square value/degree of freedom = 2.00", "Root Mean Square Error of Approximation (RMSEA) = 0.082", "Standardized Root Mean Square Residual (SRMR) = 0.087", "Comparative Fit Index (CFI) = .91" and "Non-Normed Fit Index (NNFI) = .90".

The maintenance scale factor loading was .36-.63 for, the management scale factor loading was .38-.63, and self-care confidence scale factor loading was .61-.78 (Figure 1).

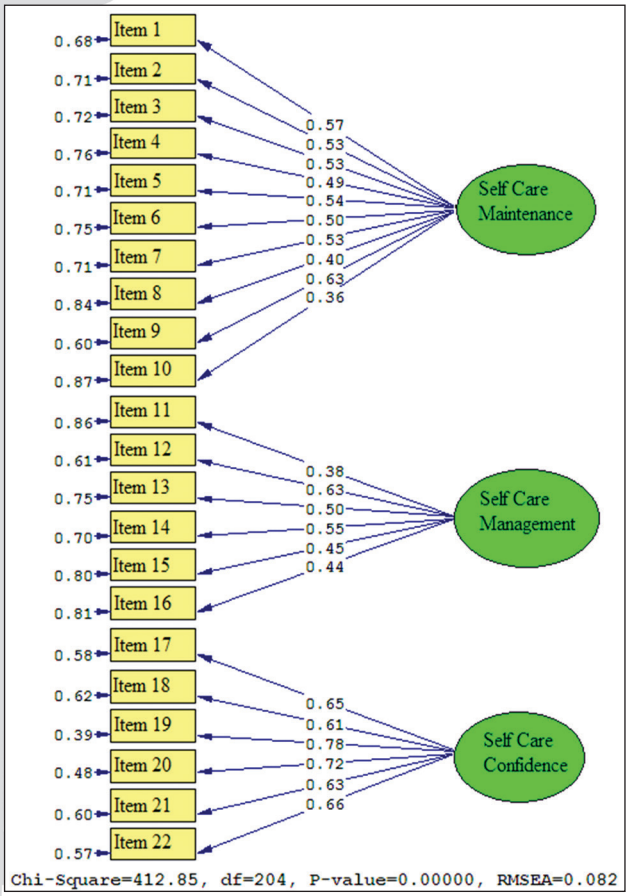


*Figure 1. Self-Care of Heart Failure Index's confirmatory factor analysis model*

### *Reliability*

### *Internal consistency analysis*

When item-total score correlations of 22 items were examined in the reliability analysis of Self-Care of Heart Failure Index, v6, it was found to be .47-.69 in the maintenance scale, .50-.71 in the management scale, .71-.83 in the self-care confidence scale and at a statistically significant level (p<.001, Table 1).

In the analysis conducted to test the internal consistency which is one of the reliability indicators of Self-Care of Heart Failure Index, v6, Cronbach alpha coefficient was .79 for maintenance scale, .63 for management scale and .85 for self-care confidence scale.

***Test-retest reliability***

To determine whether or not there were differences in the mean scores obtained from the scale between the first and second administration, the scale was evaluated using the t-test in dependent groups. No statistically differences were found ($p > .05$, Table 3).

When the relationship between scores obtained from first and second administration was evaluated with Pearson correlation analysis, it was determined that there was a very strong, positive and statistically significant relationship between test-retest scores ($p < .001$, Table 3).

## Discussion

If a scale will be used in a different language, it must display the same reliability and validity characteristics as its original form. Thus, validity and reliability of SCHFI v6 needed to be evaluated as it would be used in the Turkish sample. The results of this study provide support for the reliability and validity of the Self-Care of Heart Failure Index, v6 to measure self-care in Turkish heart failure patients.

***Validity***

The use of Confirmatory Factor Analysis (CFA) is recommended in examining the construct validity in the scale adaptation studies to test an existing hypothesis regarding the structure of items in the scale, compare the factor structure of the adapted scale to the original factor structure and evaluate similarities and differences[14].

In this study, it was determined at the end of CFA that factor loads of all items were between .36 and .78 (Figure 1). CFA recommends that each item should have a model-data fit coefficient value of at least .30 and above[16]. Thus, it was observed in this scale study that model-data fit was sufficient. Goodness of fit statistics should also be at the desired level in the confirmatory factor analysis. In the chi-square test performed as the fit statistic, it was determined that chi-square fit value was significant. Therefore, chi-square value was divided into degree of freedom and a second calculation was made. The second value was found as 2.00. The fact that this value is two or less means that it is a good model. However, the fact that this value is five or less shows us that the model has an acceptable goodness of fit[16]. It was observed in the study that model-data fit was sufficient.

The other frequent tests used to measure goodness of fit are Root Mean Square Error of Approximation (RMSEA), Standardized Root-mean-Square Residual (SRMR), Comparative Fit Index (CFI), Non-Normed Fit Index (NNFI)[16,17]. The fact that RMSEA is equal to or less than 0.080 and p value is lower than .05 (that it is statistically significant) means a good fit[16,17] while a value equal to or less than 0.10 indicates a poor fit[16]. In this study, the fact that RMSEA value was significant at .082 showed that fit was acceptable.

A value of SRMR lower than .10 and CFI, NNFI values equal to or more than 0.90 indicate that there is fit in the scale[16, 17]. In this study SRMR, CFI and NNFI values indicated a good fit. CFA results of the scale in the study conducted by Riegel et al**.** (2009) were CFI=.72, NFI=.55 and RMSEA=.07[11]. Results of this study support the construct validity of Turkish version of the SCHFI v6 and that is a valid instrument for use in Turkish samples.

***Reliability***

In this study, the SCHFI, v6 scale demonstrated acceptable internal consistency. One of the methods used to evaluate the internal consistency in the adapted scales in terms of reliability is the item analysis. Even though sufficiency level of item-total score correlation coefficients displays variety in the literature, in general, minimum level is accepted as .20, items with reliability coefficients between .30 and .40 are considered as "good" while items having reliability coefficients above 40 are reported as ideally distinctive and thus reliable[14,19]. In this study, item-total score correlation coefficients were .47 and above.

One of the methods recommended to evaluate the internal consistency is Cronbach Alpha reliability coefficient. If Cronbach Alpha coefficient is lower than .40, measurement tool is not reliable; if it has a cronbach alpha coefficient between .40

and .59, it has a low reliability; if it is between .60 and .79, it is regarded as relatively reliable while if Cronbach Alpha value is between .80 and 1.00, the scale is considered as highly reliable[12]. In our study, Cronbach Alpha coefficient of the scale was found to be within reliable limits (minimum =.63 and maximum=.85).

Cronbach Alpha coefficients of the subscales in the study conducted by Riegel et al.(2009) were .55, .59 and .82 in the self-care maintenance subscale, self-care management subscale and self-confidence in practicing the self-care activities subscale, respectively[11].

Test-retest analysis is one of the most frequently used reliability analyses and evaluates the invariance characteristic of the measurement tool. Obviously, there was consistency between measurements performed at specific intervals as there was not difference between test-retest score averages, test-retest reliability coefficient was above .70 and there was a statistically positive and highly significant relationship between test-retest scores[13,19]. The Turkish SCHFI v6 was found to have a high level of reliability.

## Conclusion

In this study where reliability and validity of the SCHFI, v6 for the Turkish society were examined, adaptation studies were conducted in accordance with international scientific methods. This study provide evidence that Turkish version of the SCHFI, is a reliable and valid instrument for assessing Turkish heart failure patients' self-care. It was concluded that the scale could be employed in the nursing practices and researches in our country.

### *Practice implications*

Evaluation of self care is important for heart failure management. No specific self care scale for heart failure patients in Turkey. This study provide evidence that Turkish version of the SCHFI, is a reliable and valid instrument for assessing Turkish heart failure patients' self-care. Thus, this scale can be used in nursing practices and researches. It could provide international comparison of results.

## References


1. *Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM et.al. American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics – 2011 Update: A Report From The American Heart Association. Circulation. 2011; 123: e18-e209.*

2. *American Heart Associations Heart Disease and Stroke Statistics 2009. ACCF/AHA 2009 Guidelines for the Diagnosis and Management of Heart Failure in Adults. A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines Developed in Collaboration With the International Society for Heart and Lung Transplantation. Available from URL: htttp://www.acc.org/clinical/guidelines/news/hf.htm (accessed 04 January 2013).*

3. *Moser DK, Watkins JF. Conceptualizing self-care in heart failure: A life course model of patient characteristics. Journal of Cardiovascular Nursing 2008; 23: 205-18.*

4. *Artinian NT, Magnan M, Sloan M, Lange P. Self-care behaviors among patients with heart failure. Heart & Lung 2002; 31: 161-72.*

5. *Dickstein K, Cohen-Solal A, Filippatos G, McMurray JJV, Ponikowski P, Poole-Wilson PA et. al. ESC Guidelines for The Diagnosis And Treatment of Acute And Chronic Heart Failure 2008: The Task Force For The Diagnosis And Treatment of Acute And Chronic Heart Failure 2008 of The European Society of Cardiology. Eur. J. Heart Fail. 2008; 933-989.*

6. *Dickson VV, Deatrick JA, Riegel B. A Typology of heart failure self care management in non-elders. Eur. J. Cardiovasc. Nurs 2008; 7: 171-81.*

7. *Riegel B, Carlson B. Facilitators and barriers to heart failure self-care. Patient Education and Counseling 2002; 46: 287-295.*

8. *Ercan I, Kan I. Reliability and validity in the scales. Uludağ Medical Journal 2004; 30: 211-216.*

9. *Gozum S, Aksayan S. Guide for intercultural scale adaptation II: Psychometric properties and intercultural comparison. Journal of Research and Development in Nursing 2002; 4(2): 9-20.*

10. *Karasar N. Data collection in: scientific research method. Ankara: Nobel Broadcast Distribution, 2000.*

11. *Riegel B, Lee CS, Dickson VV, Carlson B. An update on the self-care of heart failure index. J. Cardiovasc. Nurs 2009; 24: 485-497.*

12. *Akgül A. Statistical analysis techniques, using SPSS in medical research. Ankara: Emek Ofset Ltd.Şti, 2005.*

13. *Polit DF, Beck CT. Generating and assessing evidence for nursing practice. London: Wolters Kluwer/Lippincott Williams & Wilkins, 2008.*

14. *Gözüm S, Aksayan S. A Guide for transcultural adaptation of the scale II: Psychometric characteristics and crossal comparison. Turkish Journal of Research and Development in Nursing. 2003; 1: 3-14.*

15. *Riegel B, Carlson B, Moser D, Sebern M, Hicks F, Roland V. Psychometric testing of the self-care of heart failure index. J. Card. Fail 2004;10: 350-60.*

16. *Harrington D. Confirmatory factor analysis. pocket guides to social work research methods series. New York, USA: Oxford University Press, 2009.*

17. *Simsek OF. Introduction to structural equality modeling: basic principles and LISREL procedure. Istanbul: Ekinoks Publication, Istanbul. 2007.*

18. *Lobiondo-Wood G, Haber J. Reliability and validity. In Nursing Research Methods, Critical Appraisal and Utilization, 5th ed (Lobiondo-Wood G & Haber J eds). Mosby, St. Louis, 2002; 311–346 .*

19. *Tavşancıl E. Measurement of attitudes and data analysis with SPSS. Istanbul: Nobel Broadcast Distribution, 2005.*

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# Iodine status of school children from a previously endemic goitrous area in Montenegro

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**Abstract**

**Introduction:** Iodine deficiency disorders are still a global public health problem In Montenegro, endemic goitre is mainly occurred in the northern part of country.

**Method:** This paper analyzes the data obtained in the framework of the Sustainable Elimination of Iodine Deficiency project which involved children aged 6-12 years in 8 primary schools from the previously endemic goitrous area. Total of 215 pupils, 108 boys and 107 girls were examined for evaluation of use of iodized salt at the household level; urine samples for examination of iodine content and determination of the volume of the thyroid. All results were compared to the results of previous study.

**Results:** Total of 101 samples (46.97%) of all surveyed households were insufficiently iodinated. Overall, 62 children had urinary iodine excretion lesser than the recommended value. Comparing the size of tyroid with WHO P97 standard populations, there were 29 or 13.48% children who had gland volume greater than P97.

**Conclusions:** Comparing iodination of salt with the results of previous study no statistically significant difference was found. Median urinary iodine excretion in observed children was in line WHO recommendations. Thyroid volume measurements showed the remnants of the previous effects of iodine deficiency in the population which places the population of the former endemic areas according to the WHO standards in the category of population with mild iodine deficiency.

**Key words:** Iodine deficiency disorders, goiter, median urinary iodine, salt iodination.

## Introduction

Health disorders caused by insufficient intake of iodine (iodine deficiency disorders - IDD) include all consequences of iodine deficiency, which can be prevented with adequate intake of iodine. IDD are still a global public health problem which affect the growth and development of millions of children worldwide. Iodine deficiency is the major cause of mental retardation that can be prevented. (1.2) According to the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) data, about 43 million people in the world are affected by some degree of mental retardation. People living in areas affected by severe IDD may have an intelligence quotient (IQ) of up to about 13.5 points below that of those from comparable communities in areas where there is no iodine deficiency. This mental deficiency has a direct effect on the learning ability of children, women's health, quality of life in communities and economic productivity. Approximately 1.9 billion people or 31% of the world's population is exposed to iodine deficiency, which is most prominent in Southeast Asia and Europe. (3,6)

For the European region is significant that nearly half of all households do not use adequately iodized salt, which is reflected in the urinary iodine excretion of school children from 6 to 12 years and it ranges from 30 mg/l in Albania to 228 mg/l in Macedonia. According to these data, in 11 European countries is registered an inadequate iodine intake with prevalence of 47.8% or 24.9 million. (4,3,7)

In Montenegro, endemic goitre is mainly occurred in the northern part of country, particularly in the valley of the River Lim, the municipalities of Bijelo Polje, Pljevlja and Rozaje. The introduction of legislation establishing mandatory iodination of salt intended for human consumption and domestic animals in the country gave very good results, prevalence of endemic goiter decreased and in the eighties of last century, none case was diagnosed as endemic cretinism. (5)

According to study performed at a representative sample of school-age children in endemic

and non-endemic areas of Montenegro, palpable enlargement of thyroid gland is found in slightly more than a quarter of students surveyed and ascertained mild iodine deficiency. (16)

To meet the daily requirements of adults for iodine which is up to 150 mg per day, with the usual intake of 10g of salt and losses (transport, cooking, storage), which amounts up to 20%, it is recommended that content of iodine in salt should be 20 - 40mgJ/kg salt. The legal requirement in Montenegro is 12-18mgJ/kg salt (2.10).

Usually in the population when tracking IDD we use indicators classified into three groups. Process indicators related to the assessment and monitoring of the salt iodination at all levels, impact indicators related to the assessment of iodine status of the population and assessment of IDD dependent on salt iodination and third groups are sustainability indicators, ie indicators to assess whether iodine deficiency has been successfully eliminated in population and to judge whether achievements can be sustained and maintained. (2,3)

Impact indicators indicate the exposure or the consequences of iodine deficiency. Iodine excretion in urine reflects iodine intake, and the consequences of iodine deficiency are morphological (size of the thyroid gland, thyroglobulin) or functional (thyroid hormones, TSH). It is known that the size of the thyroid gland in a given population can not return to normal for at least months after correction of deficit in iodine intake. (4,12)

In contrast to the size of the thyroid gland which is changing slowly, the amount of iodine in the urine is a good indicator of the previous daily intake and is recommended as an indicator to evaluate the iodine status of the population. Urinary excretion of iodine is relatively easily accessible, acceptable and internationally recommended method for assessing iodine status of the population, because all the required steps in obtaining valid data for population studies are established and further developed. (2,3)

Ultrasonic measurement of the gland is precise, safe and non-invasive method. There are recognized international standards for thyroid volume in school children as a function of gender, age and body surfaces that define the population with adequate iodine intake.

School children are the best age group for monitoring IDD in the population, because they are easily accessible, sensitive and a good indicator of the current status of IDD in the population. All indicators of IDD can be tested in this age group. Existing standards of the World Health Organization (WHO) for the volume of the thyroid gland are related to the 6-12 age group. According to the WHO recommendation, median urinary iodine concentration of 100μg and higher define population which is not in iodine deficiency, i.e. at least 50 per cent of the samples should be above 100 mg/l, while less than 20% of the samples may have less than 50μg / l. (2)

Adequate iodine intake in pregnant women is defined by median urinary iodine excretion between 150249 mg/l of urine. At values higher than 300 mg/l urine, there is a risk of adverse effects of hyperthyroidism. (14, 15)

Epidemiologic criteria to evaluate the severity of IDD in a given area is based on the prevalence of goiter in schoolchildren. Total goiter rate - TGR is taken as a parameter that determines the severity of IDD. TGR presents the number of subjects with goiter grade 1 and 2 divided by the total number of respondents, expressed in percentages. Level of 5% or more of goiter in children aged 6-12 years is perceived as a public health care problem. It must be pointed out that the TGR of 5% may be caused by other exogenous factors, not just iodine deficiency. TGR greater than 30.0%, define population with severe IDD. (2,13)

## Aim of study

To examine whether the population living in former endemic area, which is the northern part of Montenegro, is at risk of iodine deficiency;

To examine whether there are differences in iodine status in former endemic area comparing the obtained results with the results of earlier studies;

To check whether the given recommendations led to the elimination of iodine deficiency in former endemic area.

## Materials and methods

This paper analyzes the data obtained in the framework of the Sustainable Elimination of Iodine Deficiency project conducted in children aged 6-12 years in 8 primary schools from the former endemic areas.

Obtained results were compared with the results of previous research that has been conducted five years earlier at the former endemic area.

Research plan was developed in accordance with the recommendations of the WHO and the International Council for the Control of Iodine Deficiency Disorders (ICCIDD).

According to methodology, 24 students per school - 12 boys and 12 girls, were observed. Each participant undergo following procedure:

1) Evaluation of the use of iodized salt at the household level.

Samples of salt in the amount of around 50g (cup of coffee), with a label on the packet, were brought by children from their homes in a clean, dry, plastic containers, marked and protected from moisture and light. Then the samples were transported to the laboratory, where analyzes were performed to determine the content of iodine in salt by standard titration method. Results of the analysis are compared with the values prescribed in the applicable regulations, as well as the recommendations of WHO and ICCIDD.

2) Taking urine samples for examination of iodine content.

Urine was collected in labeled containers intended for this purpose. After collection, the samples were subjected to freezing, and then delivered to the laboratory. Urinary iodine excretion was determined by a method based on chloric acid digestion and colorimetric determination of iodine, and reduction in ceric ammonium sulfate. Obtained values of excreted urine are expressed in micrograms of iodine per liter (mg/l) of urine. Obtained results were used to calculate the median, which is then compared with a WHO and ICCIDD recommendations for populations with adequate iodine intake.

3) Determination of the volume of the thyroid gland.

The study used ultrasound with linear probe frequency of 7.5 MHz. This method is used to determine the maximum width (b), thickness of the lobe (v), transverse section and the length (a) longitudinal cross section, especially for the left and right lobe of the gland. Volume of the thyroid gland is obtained by summing the volume of the left and right lobe, which is calculated by the formula: V = axbxvx 0.479 (0.479 is an empirically determined correction factor). Gland volume is expressed in milliliters - ml. The volume of the thyroid was presented as mean, standard deviation, median and P97-the upper limit of normal in relation to age, gender and body surface area. The obtained values was compared with standard values given by the WHO for the population of school children 6 - 12 years.

Criteria for determination of iodine deficiency are: goiter prevalence above 5% and the median urinary iodine excretion less than 100 mg/l of urine.

The obtained results are compared with the results of previous studies of disorders caused by lack of intake of iodine from the former endemic areas of Montenegro in 5 years interval. That study included 170 school children 7-12 years of age from selected elementary schools in the former endemic areas.

All results were analyzed using Excel software statistical package.

Testing differences in the size of the thyroid gland and urinary iodine excretion between boys and girls was performed using statistical analytical methods for testing the significance of the difference.

## Results

Total of 215 students, 108 boys and 107 girls were examined. Age distribution was the following: 6 yrs.- 16 boys and 16 girls,7 yrs.- 19 boys and 20 girls,8 yrs. -18 boys and 18 girls, 9 yrs.-18 boys and 17 girls, 10 yrs.-19 boys and 18 girls, and at the age of 11 yr. 18 boys and 18 girls.

KJ concentration in salt obtained from households ranged from 2.3 to 32.3 mgKJ/kg of salt, and the median was 16 mgKJ/kg salt. Total of 101 samples (46.97%) of all surveyed households had a lower concentration of KJ in comparison to the standardized amount (16-24 mgKJ/kg). Only 11 samples (5.11%) have a higher concentration of potassium iodide than the recommended upper limit.

Urinary iodine excretion ranged from 25.3 to 361 μgJ/l urine, with a median 138.6 μgJ/l. Overall, 62 children had urinary iodine excretion less than the recommended value. Only six children, four boys and two girls, or 2.8% had urinary excretion over 300 μgJ / l urine. Table 1 shows the urinary excretion by gender.

*Table 1. Urinary excretion by gender*

| Iodine content in µg/l urine | urine samples | | | | | |
|---|---|---|---|---|---|---|
| | boys | | girls | | total | |
| | N | % | N | % | N | % |
| Below 20 | / | / | / | / | / | / |
| 20 to 49 | 3 | 2,78 | 10 | 9,35 | 13 | 6,04 |
| 50 to 99 | 20 | 18,52 | 29 | 27,10 | 49 | 22,79 |
| Over 100 | 85 | 78,70 | 68 | 63,55 | 153 | 71,17 |
| Total | 108 | 100 | 107 | 100 | 215 | 100 |

Observed by gender, boys had median urinary excretion 153.1 µgJ/l urine and girls 120.1 µgJ/l urine. Total 36.45% of girls and 21.30% of boys had lower excretion than is recommended. The girls had statistically poorer urinary excretion than boys when they were tested with statistical analytical method for testing the significance of the difference (Chi square 6.1).

Size of the thyroid gland in children depending on their age ranged from 1.52 ml to 14.17 ml. Thyroid volume in milliliters, arithmetic mean (AS ) and standard deviation (SD) distributed by children age and gender are given in the following table number 2.

Table 2. Arithmetic mean (AS) and standard deviation (SD) of thyroid volume distributed in relation to age and gender

*Table 2. Arithmetic mean (AS) and standard deviation (SD) of thyroid volume distributed in relation to age and gender*

| Age | Gender | | | | | |
|---|---|---|---|---|---|---|
| | boys | | girls | | total | |
| | AS | SD | AS | SD | AS | SD |
| 6 | 2,73 | 0,80 | 2,72 | 0,74 | 2,72 | 0,76 |
| 7 | 2,67 | 1,01 | 2,83 | 1,57 | 2,76 | 1,31 |
| 8 | 2,98 | 0,73 | 3,19 | 0,79 | 3,08 | 0,76 |
| 9 | 3,40 | 0,85 | 3,17 | 1,12 | 3,29 | 0,98 |
| 10 | 3,21 | 0,71 | 3,96 | 1,26 | 3,57 | 1,07 |
| 11 | 4,17 | 0,98 | 4,74 | 2,47 | 4,46 | 1,87 |
| Total | 3,20 | 0,98 | 3,44 | 1,59 | 3,32 | 1,32 |

When comparing the size of the thyroid gland between boys and girls by age, it can be concluded that there were no statistically significant differences, except at the age 10 years, where girls had significantly higher thyroid volume than boys of the same age. (t6 = 0.04, DF30, t7 = 0.37, DF 37 t8 = 0.80, t9 = 0.66 DF34, DF33 t10 = 2.18; DF35, $p < 0.05$, t11 = 0.89 , DF34).

Median volume of the thyroid by age and gender of children is displayed in Table 3.

*Table 3. Median volume of thyroid in milimeters in relation to age and gender*

| Age for both genders | 6 | 7 | 8 | 9 | 10 | 11 | 6-11 |
|---|---|---|---|---|---|---|---|
| Median for both sexes | 2,83 | 2,47 | 2,96 | 3,05 | 3,45 | 4,13 | 3,12 |
| Median for boys | 2,78 | 2,51 | 2,85 | 3,31 | 3,20 | 3,97 | 3,02 |
| Median for girls | 2,87 | 2,25 | 3,21 | 2,91 | 4,05 | 4,23 | 3,20 |

Given that WHO has standardized values for the thyroid in children 6 to 12 years by gender, these are used for comparison and evaluation of the prevalence of endemic goiter in the population. The distribution of the examined children with values greater than the upper limit, P97, by age and gender is given in the following table 4.

Comparing the size of the gland with WHO P97 by age and gender standard populations, there were 29 or 13.48% children who had gland volume greater than P97 for a given age and gender. In boys there were 13 (12.03%), while in girls 16 (14.95%).

***Results from previous study which can be used for comparison with the obtained results***

The previous study included 170 school children, 87 boys and 83 girls, aged 7-12 years from selected elementary schools from the former endemic areas.

In relation to the age distribution was the following:

At the age of 7 there were 10 boys and 8 girls, at age of 8 - 19 and 26, at age of 9 - 19 and 14 girls,

*Table 4. Number of children, whose thyroid exceed P97 for age and gender*

| Age | Value P97(ml) boys | ValueP97(ml) girls | Proportion boys | Proportion girls |
|---|---|---|---|---|
| 6 | 2,91 | 2,84 | 4 | 4 |
| 7 | 3,29 | 3,26 | 2 | 3 |
| 8 | 3,71 | 3,76 | 3 | 4 |
| 9 | 4,19 | 4,32 | 2 | 1 |
| 10 | 4,73 | 4,98 | 0 | 3 |
| 11 | 5,34 | 7,73 | 2 | 1 |

*Table 5. Urinary excretion of iodine in relation to gender*

| Iodine content in µg/l urine | Urine samples | | | | | |
|---|---|---|---|---|---|---|
| | boys | | girls | | total | |
| | N | % | N | % | N | % |
| below 20 | 0 | 0.00 | 5 | 9.09 | 5 | 4.42 |
| 20 to 49 | 7 | 12.07 | 8 | 14.55 | 15 | 13.27 |
| 50 to 99 | 22 | 37.93 | 13 | 23.64 | 35 | 30.97 |
| abow 100 | 29 | 50.00 | 29 | 52.73 | 58 | 51.33 |
| total | 58 | 100.00 | 55 | 100.00 | 113 | 100.00 |

at age of 10- 20 and 18, and at the age of 11 - 19 boys and 17 girls were examined.

KJ concentration of salt in households was determined in thirty-one sample. In 11 (35.48%) of the samples, there was insufficient concentration of KJ, in 16 (51.61%) samples salt iodination was adequate, while in 4 samples KJ concentration was higher than prescribed.

Urinary iodine excretion is controlled in a total of 113 students, 58 boys and 55 girls.

The values of urinary excretion of iodine in the study population ranged from 1 mg/l to 314 mg/l and the median was 100 mg/l.

The values of urinary iodine excretion in relation to gender are shown in Table 5.

Twenty students (17.70%) had urinary iodine excretion less than 50 mg/l urine, while 58 students (51.33%) had a value of iodine excretion greater than 100µg/l. Five female students had urinary excretion of iodine less than 20 mg/l.

There is statistically significant difference in the level of urinary excretion between boys and girls - girls had poorer urinary iodine excretion, (Chi-square = 7.3, $p < 0.05$).

The volume of the thyroid = is considered in relation to gender and age of subjects, and then expressed with the average value (arithmetic mean-AS) and standard deviation (SD). The results are presented in Table 6.

*Table 6. Aritmetic mean (AS) and standard deviation (SD) of thyroid volume (expressed in ml) in relation to the age and gender*

| Age | Gender | | | | | |
|---|---|---|---|---|---|---|
| | boys | | girls | | total | |
| | AS | SD | AS | SD | AS | SD |
| 7 | 2.32 | 0.51 | 1.91 | 0.66 | 2.13 | 0.60 |
| 8 | 2.55 | 1.06 | 2.35 | 0.67 | 2.43 | 0.85 |
| 9 | 2.67 | 0.72 | 2.68 | 0.60 | 2.64 | 0.66 |
| 10 | 2.91 | 1.20 | 3.05 | 0.86 | 2.99 | 1.04 |
| 11 | 3.23 | 0.98 | 3.26 | 0.95 | 3.24 | 0.95 |

Average value ranged from 1.91 ml in girls aged 7 years to 3.26 ml in girls aged 11. Statistically significant difference in the size of thyroid gland between boys and girls of the same age did not appear when testing significance of differences

$t(7) = 1.41, p > 0.05,$
$t(8) = 0.77, p > 0.05;$
$t(9) = -0.04, p > 0.05,$
$t(10) = -0.4, p > 0.05,$
$t(11) = -0.09, p > 0.05;$

Median volume of thyroid by age are presented in Table 7

*Table 7. Median volume of thyroid gland in relation to the children age*

| Age | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|
| Median in ml | 2.10 | 2.32 | 2.56 | 2.72 | 3.36 |

***Comparison of results from two studies***

Comparing the size of thyroid gland by testing the difference in arithmetic means in these two studies, it is concluded that there is no statistically significant difference in the size of the glands in boys aged 7, 8 and 10 years. There was a statistically significant difference in the age of 9 and 11, in gland volume - the boys in recent study have a slightly higher volume than in the previous study ($t7 = 0.99$, df 27, for $t8 = 1.38$, df 35, $t10 = 0.93$, df 37 $p > 0.05$, $t9 = 2.75$, DF 35 and $t11 = 2.76$, df 33 $p < 0.01$).

For girls of the same age of 7 or 9 years there were no statistically significant differences, $t7 = 1.56$, DF 26, $t9 = 1.44$, DF, $p > 0.05$, while for a girls of 8, 10 and 11 years old, there was a statistically significant difference in the volume of thyroid gland. Girls in our study have more voluminous glands than in the earlier study, $t8 = 3.65$, df 42, $t10 = 2.45$, DF 34 and $t11 = 3.08$, df 52, $p < 0.01$,

Comparing iodination of salt samples brought in by children from their homes, in relation to respective values from previous research, it can be concluded that there is no statistically significant difference in salt iodination (Chi square is 1.44, $p > 0.05$).

Comparing urinary iodine excretion in children from these two studies in relation to the recommended value (100 mg/l), it is concluded that urinary iodine excretion was better than in previous research (Chi square was 12.70, $p < 0.01$).

**Discussion**

Health disorders caused by insufficient iodine intake were serious public health problem in the past. Severe health disorders and endemic cretinism are rapidly eliminated by introducing mandatory iodine prophylaxis, while occurrence of milder forms of goiter in Montenegro has not yet been eliminated.

Ten years ago, research was carried out on representative sample of school children in endemic and non-endemic areas of Montenegro in the framework of the project "Endemic goitre in Montenegro and iodine prophylaxis." Thyroid gland enlargement on palpation test was determined in 27.3% of the surveyed participants. Thyroid enlargement was more common 37.5% in children from the north of the country, children from rural areas and among girls. Median urinary excretion was 8.23 mg/dl, which according to accepted criteria represent mild iodine deficiency. Iodination of salt was not adequate in the samples taken from the market, households and the production - 36.7% of all samples have less than 16mg KJ per kg of salt. (16)

Recent research, which covered only the former endemic area demonstrated no endemic goiter, but according to the criteria of WHO and ICCIDD, the existence of mild iodine deficiency cannot be excluded with certainty, whereas the degree of salt iodination in former endemic areas has not been satisfactory.

This survey included 215 participants, aged 6-12 years, nearly homogeneously distributed by sex and age. Research has shown that the degree of salt iodination in samples from household does not meet legal or WHO criteria that are needed to provide adequate levels of iodine in the population. Total of 101 (46.97%) of salt samples from children households have a lower concentration than the standardized amount which is 16-24 mgKJ/kg salt. Only 11 samples or 5.11% has a higher concentration of potassium iodide than the upper limit specified by the regulations.

According to urinary iodine excretion examined population have sufficient iodine intake. Urinary iodine excretion ranged from 25.3 to 361 μgJ/l urine, with a median 138.6 μgJ/l, which is consistent with results for the population in the United States. (17) and better than in the UK and New Zealand. (18, 19)

Total 28.84% of the children had urinary iodine excretion lower than the recommended value. Only six children, four boys and two girls (2.8%) had urinary excretion over 300 μgJ/l urine, which places them at risk category for developing hyperthyroidism.

By analyzing the size of the gland can be stated that thyroid in children increases with age and is highest in the age of 11 years. While comparing the size of thyroid between boys and girls by age, it can be concluded that there are no significant differences, except at the age of 10, where the girls have a thyroid significantly larger than boys of the same age.

Comparing the size of the gland with the upper limit of normal, i.e. P97 per age and gender of the WHO standard population, which are given in Table 8, it can be stated that 29 (13.48%) of children have gland volume greater than P97 for the same age and sex. Among boys, 13 (12.03%), while for girls there were 16 (14.95%). It can be stated that the population of school children in former endemic area is in mild iodine deficiency according to this criteria.

*Table 8. P97 thyroid volumes of WHO standard population*

| Age | P97 Boys | P97Girls |
|---|---|---|
| 6 | 2,91 | 2,84 |
| 7 | 3,29 | 3,26 |
| 8 | 3,71 | 3,76 |
| 9 | 4,19 | 4,32 |
| 10 | 4,73 | 4,98 |
| 11 | 5,34 | 5,73 |
| 12 | 6,03 | 6,59 |

*Source: WHO, 2007.*

Comparing the presented results obtained from the two studies in former endemic area can be concluded that there are no significant differences in salt iodination, or in other words there is a presence of inadequate amounts of iodine in salt in the studied households.

Comparing urinary iodine excretion in children in these two studies with the recommended values of 100 mg/l, it can be concluded that urinary iodine excretion was significantly better in this than in the previous survey.

Comparing the size of thyroid, that is testing the significance of mean differences in these two studies, it can be concluded that there is no statistically significant difference in the size of the glands in boys aged 7, 8 and 10 years. At the age of 9 and 11 years, the boys have a slightly higher volume of the gland than in the previous survey. For girls of the same age of 7 or 9 there were no statistically significant differences, while at the age of 8, 10 and 11 years old, there was a statistically significant difference in the sense that girls had a higher volume of thyroid than in the earlier study.

**Conclusion**

It can be concluded that iodine is homogeneously distributed in the salt, but his concentrations are lower comparing to the recommended values. In the course of study in selected households neither of them used non-iodized salt. Almost half of the salt samples (46.97%) originating from households have not been adequately iodized. WHO criteria, which says that 90% of salt samples from households should be adequately iodized as a condition for the prevention of iodine deficiency, is not met in this studied population.

Median of urinary iodine excretion in school children, as the most important indicator of iodine intake in the population is 138.6 μgJ/l of urine which is in line with WHO recommendations, or by this criteria this population have sufficient iodine intake.

Comparing iodination of salt with the results of previous study it can be concluded that the situation is similar, or there is no statistically significant difference. Urinary iodine excretion was significantly better than in the previous period.

Since the urinary excretion of iodine in the population is satisfactory, and that households use lower iodinated salt than recommended, probably a very significant source of iodine in the diet is iodine from salt added to food during the technological process of production e.g. production of bread, pastries, cheese, processed meat etc.

Thyroid volume measurements showed the remnants of the previous effects of iodine deficiency in the population, i.e., it was concluded that a total of 12.03% boys and 14.95% girls or 13.48% of children have thyroid volume larger than WHO standards which are used for children with adequate iodine intake. This places the population of the former endemic areas according to the criteria in the category of population with mild iodine deficiency.

The volume of thyroid in boys age 9 and 11 and girls age 8, 10 and 11 is higher comparing with peers from previous research, which indicates that the effectiveness of the undertaken public health measures must be reviewed, because they haven't reached their goals.

***Proposed measures***

It is necessary to consistently carry out iodine prophylaxis at all levels in order to efficiently and sustainable eliminate and eradicate all health problems caused by insufficient iodine intake

Given that there is inadequate iodination of salt, and for iodination is mostly used unstable potassium iodide which content in salt can easily be decreased through the process of oxidation and iodine evaporation in current climate conditions, in order to achieve the WHO recommendation it is necessary to consider that produced or imported salt in Montenegro should be iodinated with potassium iodate, as far more stable form, in amount recommended by the WHO, taking an average of 20 mg of iodine per kg of salt.

In the future, it is necessary to continuously control the quality and degree of salt iodination on Montenegro market, and in particular to intensify control of manufacturing plants in Montenegro to ensure their uniformity of iodination.

It is necessary to carry out continuous health education campaign in schools and in the media in order to improve knowledge on the effects of inadequate iodine intake and to give practical advice about how to mitigate the loss of iodine from salt in households.

In cooperation with gynecologists it is necessary to make a an educational program about nutrition of pregnant women and especially on adequate iodine intake due to its great importance in embryonic and fetal development.

Given the fact that urinary iodine excretion is the most important indicator of iodine intake in the population it would be necessary to control the level of urinary excretion of pregnant women every year and in children age 6-12 every two years.

## References


1. *De Benoist B, McLean E, Andersson M, Rogers L. Iodine deficiency in 2007: global progress since 2003. Food Nutr Bull, 2008; 29(3): 195-202.*
2. *WHO. Assessment of Iodine Deficiency Disorders and Monitoring their Elimination: A Guid for programme menagers. WHO, Geneva, 2007.*
3. *WHO. Iodine deficiency in Europe: A continuing public health problem WHO, Geneva, 2007*
4. *Delange F, Hetzel BS. The iodine deficiency diserdors. Hennemann G, DeGroot L, eds. Endocrine Education, inc., 2003. Thyroid Manager http: //www.thyroidmanager.org/Chapter20/chapter20.pdfaccessed20 September 2005)*
5. *Zavod za zdravstvenu zaštitu Podgorica. Endemska struma u Crnoj Gori i jod profilaksa, Podgorica: ZZZP; 2001.*
6. *Bleichrodot N, Born MP. A metaanalysis of research on iodine and its relationship to cognitive development. In Stanbury JB, ed The damaged brain of iodine deficiency. New York, Cognizat Communication, 1994; 195-200*
7. *Karanfilski B, et al. Macedonia success IDD elimination story. Skopje, Institut of pathoshysiology, Nuclear Medicine and Medical Faculty, 2004.*
8. *Zimmermann MB, et al. Iodine Deficiency disorders Rewiew Article The Lancet, 4-10 October 2008; volume 372, Issue 9645: 1251-1262*
9. *Zimmermann M, et al. Iodine deficiency, Endocr. Rev.2009; 30: 376-408*
10. *Pravilnik o kvalitetu i drugim zahtjevima za so za ljudsku ishranu i proizvodnju namirnica. Sl. list SCG br.31/2005*
11. *Patrick L. Iodine: deficiency and therapeutic considerations. Altern Med Rev, 2008; 13(2): 116-127.*
12. *Rasmussen LB et al. Relation between various measures of iodine intake and thyroid volume, thyroid nodularity, and serum thyroglobulin. American Journal of Clinical Nutrition, 2002; 76: 1069-1076.*
13. *Network for sustained elimination of Iodine Deficiency Country review Guidelines. New York, United Nations Children s Fund, 2006.*
14. *WHO. Reaching optimal iodine nutrition in pregnant and lactating women and young children. World Health Organization. Geneva (In Press).*

15. *Iodine status in late pregnancy and psychosocial determinants of iodized salt use in rural northern Viet Nam Bulletin of the World Health Organization 2011; 89: 813-820. doi: 10.2471/BLT.11.089763*

16. *Žižić Lj. i dr. Jodni status školske djece u Bijelom Polju, Zavod za zdravstvenu zaštitu Podgorica, Podgorica, 2003.*

17. *Lee S, et all. Iodine Deficiency emedicine.medscape. com/.../122714-overvie 2012.*

18. *Vanderpump MPJ, Lazarus JH, Smyth PP, Laurberg P, Holder RL, Boelaert K, Franklyn JA. Iodine status of UK schoolgirls: a cross-sectional survey Lancet 2011; 377: 2007-2012*

19. *Skeaff S, et all. A comprehensive assessment of urinary iodine concentration and thyroid hormones in New Zealand schoolchildren: a cross-sectional study Nutrition Journal www.nutritionj.com/content/11/1/31*

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# Malignant wound management in breast cancer patient

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**Abstract**

**Introduction**: A malignant wound is usually presents as a discrete non-tender nodule, and often accompanied with malodour. Approximately 62% of malignant wounds will develop in the area of the breast and 24% in the head and neck area. It is estimated that 5-10% of patients with metastases will develop malignant wounds, and they primarily occur in the last 6 months of life. These wounds are not expected to heal unless the pathology is controlled. The wound management is focused on controlling and/or eliminating the distressing symptoms associated with these wounds.

**Case report**: A 40 year old Malay female is presented with left breast lump for 7 months and ulceration accompanied with pain at the swelling for 2 weeks. Chest x-ray findings show a large soft tissue shadow on left breast but no bony lesion. There were ulceration, irregular surface, necrotic area, pus discharge and foul smelling at left breast. Hard swelling was occupying the whole left breast but there was no nipple discharge. Patient is diagnosed with locally advance left breast cancer, $T_4N_1M_X$ and is planned for toilet mastectomy.

**Discussion**: Bactigras is used as dressing for the malignant wound on patient's left breast. Bactigras is a low adherent dressing and contains chlorhexidine which is an antimicrobial against a wide range of Gram-positive and Gram-negative bacteria. Activated charcoal and metronidazole powder are used for dressing of the malignant wound in this patient. Activated charcoal is effective in controlling the wound malodour as it attracts and binds to the volatile odour causing molecules, thus preventing their escape from the local wound area. For infected wound with odour, topical metronidazole is used as an antimicrobial dressing to reduce or eliminate the malodour.

**Conclusion:** Although palliative care of the patient with a malignant wound will not cure patients of their advanced cancer, it still serves as an important component to patient as it improves quality of life and eases their pain.

**Key words:** Antimicrobial Dressing, Breast Cancer, Malignant Wound Management.

## Introduction

A malignant wound is also known as tumor necrosis, a fungating wound, ulcerating cancerous wound, or malignant cutaneous wound. It is usually presents as a discrete non-tender nodule, skin toned, pink, violet-blue or black brown in color and often accompanied with large amounts of necrotic material which account for the odour.

Malignant or fungating wound is caused by infiltration of the epidermis by primary or metastatic tumor. After the formation of fungating wound, alteration of the tissue perfusion and expansion of mass occurred, this leads to tumor hypoxia and necrosis. To support the growth of the tumor, growth factors are secreted by additional tumor cells.

Approximately 62% of malignant wounds will develop in the area of the breast and 24% in the head and neck area.[1] It is estimated that 5-10% of patients with metastases will develop malignant wounds, and they primarily occur in the last 6 months of life.[2] Malignant wounds can occur with many types of cancers, commonly associated with skin, breast, lung, head and neck, melanoma, colorectal, sarcomas, cervix and ovarian cancers.

These wounds are not expected to heal unless the pathology is controlled. Most of malignant wounds will continue to deteriorate over time and can be devastating to patient's well-being, both physically and emotionally. The approach to care is holistic and primarily palliative with the aim to

control symptoms at the wound site and reduce the impact of the wound on the patient's daily life. Hence, the wound management is focused on controlling and/or eliminating the distressing symptoms associated with these wounds.

## Case report

A 40 years old Malay female is presented with left breast lump for 7 months and ulceration accompanied with pain at the swelling for 2 weeks. Patient was apparently well since 7 months ago when she started to note that she had lump at her left breast. Initially, the lump is small in size, which is about thumb size. Fine needle aspiration cytology was taken in November. Since then, the lump started to increase in size progressively, to the current size.

Patient also complaint of ulceration over the swelling since 2 weeks ago accompanied with pain, foul smelling, pus discharge, and blood discharge. There is history of blood discharge from nipples, but no history of hemoptysis, jaundice or body tenderness. Patient has no other medical illness and has no family history of malignancy. Patient has history of taking traditional medication for body ache and defaulted follow up since January 2012 because she is not keen for surgical intervention so she went for traditional healer instead.

Patient is a housewife with 4 children. She attained menarche since 12 years old and last menstrual period is about 2 months ago. Last child birth was 5 years ago and she breastfeed each child for about 2 to 3 months. Chest x-ray findings show clear lung and no pleural effusion (Figure 1). There is a large soft tissue shadow on left breast but no bony lesion.

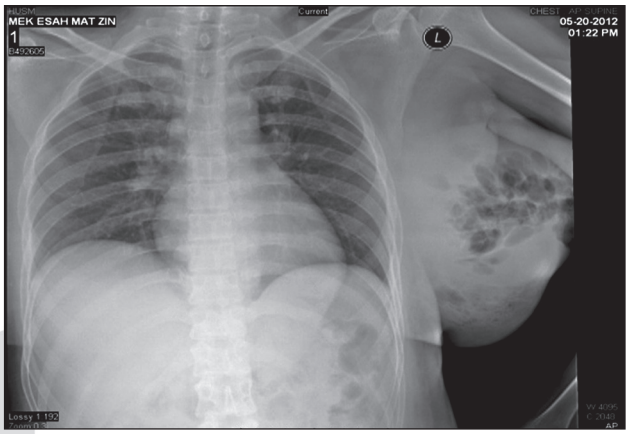


*Figure 1. Chest x-ray of breast cancer patient*

Physical examination showed patient appeared conscious and alert. Blood pressure was 100/66 mmHg with pulse rate of 106 beats per minute and temperature of 37ºC. Lungs were clear and abdominal wall was soft and non-tender. There were ulceration, irregular surface, necrotic area, pus discharge and foul smelling at left breast. Hard swelling was occupying the whole left breast but there was no nipple discharge. Patient mobility is restricted. Patient is diagnosed with locally advance left breast cancer, $T_4N_1M_X$ and is planned for toilet mastectomy. Detail Patient's treatment include in Table 1.

## Discussion

In this patient, Bactigras is used as dressing for the malignant wound on her left breast. Bactigras is a low adherent dressing that are cheap and widely available which allow exudate to pass through into a secondary dressing while maintaining moist wound bed.[3] It is manufactured in the tulles form, which are open weave cloth soaked in soft

*Table 1. Drug Treatment in Ward*

| Drug | Dose | Indication |
|---|---|---|
| Tab. Metronidazole (Flagyl) | Tablets are crushed and smeared on ulcer during dressing | For dressing with normal saline and Bactigras to control wound malodour and infection |
| Tab. Activated charcoal | | |
| IV Tramadol (Tramal) | 50 mg thrice daily ( for 2days) | For pain management |
| Cap. Tramadol | 50 mg thrice daily | For pain management |
| Cap.Celecoxib (Celebrex) | 200 mg twice daily | For pain management |
| Tab. Ferrous fumarate | 200 mg once daily ( for 2days) | Hematinics |
| Tab. Folic acid | 5 mg once daily | Hematinics |
| B-complex, vitamin C | Once daily | Hematinics |
| Cap. Cloxacillin | 500 mg four times daily | To control wound infection |
| Ravin enema | A single dose | To promote bowel movement |
| Tab. Pantoprazole | 40 mg twice daily | For prophylaxis of stress-induced ulcer |

paraffin or chlorhexidine. It is designated to reduce adherence at the wound bed and to improve its low adherence properties, normal saline dressing is also used in this patient. Bactigras dressing contains chlorhexidine which is an antimicrobial against a wide range of Gram-positive and Gram-negative bacteria, including Methicillin-resistant Staphylococcus aureus (MRSA), a commonly found bacteria strain in hospital setting.[4]

Activated charcoal and metronidazole powder are used for dressing of the malignant wound in this patient. Activated charcoal is effective in controlling the wound malodour as it attracts and binds to the volatile odour causing molecules, thus preventing their escape from the local wound area.[1] The odour is caused by bacterial infection and is probably the most distressing symptom for patient as it may trigger vomiting reflexes and also cause embarrassment, disgust as well as social isolation. Activated charcoal loses its odour-adsorbing properties when it becomes wet, hence it is often necessary to change the dressing frequently.[5]

For infected wound with odour, topical metronidazole is used as an antimicrobial dressing to reduce or eliminate the malodour. According to a randomized, placebo-controlled, double-blind study conducted by Bale and colleague[6], it was found that metronidazole gel led to a faster reduction in odour compared to placebo, with 100% effectiveness compared to placebo with only 76% effectiveness at eliminating odour within 3 days. In a prospective study, metronidazole gel 0.75% was used as a daily application to wounds after cleaning with sterile saline 0.9%.[7] The findings showed that on day 0, 64% of wounds were rated odorous but on day 7, it was reduced to 11% and on day 14, to 4%. Besides that, the bacterial colonization of the wounds on swab results was also improved. This result thus supports the use of metronidazole to control wound malodour and reduce bacterial infection. In another small study involving 5 women with ulcerated tumours, metronidazole gel 0.8% which prepared by the investigators was applied topically to the surface of ulcerated tumours once or twice daily. Subjective assessment showed that four out of five people had elimination of odour while one of them had improvement in odour after 2-5 days of topical metronidazole. Anaerobic bacterial colonies were reduced after application of metronidazole on the ulcerated tumours.[8]

## Conclusion

Although palliative care of the patient with a malignant wound will not cure patients of their advanced cancer, it still serves as an important component to patient as it improves quality of life and eases their pain. A suitable low adherent dressing with combination of topical activated charcoal and metronidazole can help to control malodour of the malignant wound.

## References


1. *Naylor W. Part 1: Symptom control in the management of fungating wounds. [Accessed 26 May 2012]. Available from: http://www.worldwidewounds.com/2002/march/Naylor/Symptom-Control-Fungating-Wounds.html*
2. *WRHA wound care recommendations. [Accessed 26 May 2012]. Available from:http://www.wrha.mb.ca/professionals/ebpt/files/WC-08MalignantWounds.pdf*
3. *Jones V, Grey JE, Harding KG. Wound dressing. BMJ 2006; 332: 777-80.*
4. *Muangman P, Nitimonton S, Aramwit P. Comparative clinical study of Bactigras and Telfa AMD for skin graft donor-site dressing. Int J Mol Sci 2011; 12: 5031-8.*
5. *Wilson V. Assessment and management of fungating wounds: a review. British Journal of Community Nursing; 2005; 10(Suppl 3): S28-34.*
6. *Bale S, Tebbie N, Price P. A topical metronidazole gel used to treated malodorous wounds. British Journal of Nursing; 2004; 13(11): S4-11.*
7. *Finlay IG, Bowszyc J, Ramlau C, Gwiezdzinski Z. The effect of topical 0.75% metronidazole gel on malodorous cutaneous ulcers. Journal of Pain and Symptom Management 1996; 11(3): 158-62.*
8. *Kuge S, Tokuda Y, Ohta M et al. Use of metronidazole gel to control malodour in advanced and recurrent breast cancer. Japanese Journal of Clinical Oncology 1996; 26(4): 207-10.*



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# Health education for hypertension in the elderly in Western Chinese villages: An intervention study

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**Abstract**

**Purpose:** To explore the effects of health education programs about hypertension (HEPH) among the elderly in western Chinese villages.

**Methods:** We conducted the HEPH from July 2011 to June 2012, including health lectures, personal consultation, peer-education and distribution of cartoon pamphlets. First, a questionnaire was delivered to 438 randomly selected elderly people from seven villages to obtain basic information concerning relevant HEPH, then the regular intervention measures about HEPH were given to the elderly for twelve months, and the resultant information concerning HEPH was obtained.

**Results:** Poor knowledge regarding the causes and preventive measures of hypertension was observed in these aged participants before implementation of the HEPH in Chinese villages. However, their overall knowledge of hypertension was significantly improved after the HEPH ($P<0.05$).

**Conclusion:** HEPH can greatly improve the elderly's knowledge of hypertension, which may help to reduce the risk of hypertension in elderly, thereby enhancing their quality of life.

**Key words:** Elderly, hypertension, health education, China.

## Introduction

With the continuous advances and improvements in living standards and an ever-increasing life span, aging has raised serious concerns worldwide (1). Hypertension, which is often found in the elderly population, is one of the leading causes of morbidity and mortality in the world, especially in China. Hypertension can lead to pathological changes in the heart, brain, kidney and other organ systems. In China, the most common complication of hypertension is stroke, followed by hypertension-related heart damage, including myocardial hypertrophy, coronary arteriosclerosis, arrhythmia and heart failure, and then kidney damage and peripheral angiopathy. In the mid-to-late stage of hypertension, retinopathy can occur. A less common but serious complication is aortic dissection. Diabetes is also a common comorbidity of hypertension. How to effectively prevent and cure hypertension has become an increasingly important concern of public health providers. If hypertension can be satisfactorily controlled and managed, the incidence of hypertension-related diseases will probably decrease remarkably, especially in the elderly population (2, 3). For this purpose, we conducted an investigation and intervention of hypertension knowledge in elderly people from several Chinese villages from July 2011 to June 2012. Meanwhile, health education was conducted in various ways including health lectures, personal consultation, peer-education approach and distribution of cartoon health pamphlets in an elderly population. The study aims at improving knowledge of hypertension in the elderly people in order to decrease the incidence of hypertension.

## Materials and methods

### *Subjects*

A total of 438 elderly people (age 60 or older), from Shaanxi province of China, including seven Villages, were randomly selected. All the subjects received a questionnaire-based survey and then underwent strict HEPH from July 2011 to June 2012.

### *Methods*

Surveys were conducted at baseline and after intervention by medical undergraduates from target University. The self-designed questionnaire used in questionnaire-based investigation was

composed of three sections, including demographic profiles, knowledge of hypertension and medical needs of the elderly. The self-designed questionnaire contained hypertension knowledge that was also used in the post-intervention questionnaire survey. The participants were required to fill in questionnaire by themselves after a thorough explanation for the items in the questionnaire by trained undergraduates before and after intervention respectively. If any subject was unable to fill the questionnaire by themselves, the undergraduates would fill the questionnaire in accordance with the opinions of the participants. Before the implementation of various forms of health education, a total of 438 questionnaires were distributed and 435 (99.32%) of them were returned. After the HEPH, one person went to Australia with her daughter and two participants died, 435 (100%) of 435 questionnaires were returned.

Three stages of the study:

- Stage 1 Collecting basic information before the HEPH
  Questionnaires were delivered to 438 randomly selected elderly people from seven villages in July 2011 to obtain the basic information concerning relevant knowledge of hypertension.
- Step 2 Performing the HEPH
  Firstly, intervention measures were given and the same HEPH was applied to elderly people in the seven villages. Finally, the questionnaires were delivered to 435 randomly selected elderly people from the same seven villages to obtain the critical information concerning relevant hypertension after the HEPH was completed in June 2012.
- Stage 3 Data analysis
  Data analysis was performed using SPSS software, version13.0. After the collection of all the valid questionnaires, chi-square test was used to compare the difference in percentages before and after intervention measures.

***Ethical considerations***

The research proposal was submitted to and approved by the Ethical Review Committee in target University. In addition, before the data collection, approval from each relevant village was obtained. All participants were informed about the purposes and the methods of the investigation and intervention. They were also informed that participation in the study was voluntary, so they could refuse to participate or withdraw from the study at any time without being penalized or subjected to economic losses. Moreover, the participants were assured that their responses would be kept confidential and their identities would not be revealed in research reports and publications of the study. Lastly, the participants who agreed to participate in the investigation were asked to sign a written consent.

***The Process of Health Education***

The HEPH included four main steps, which were performed by medical teams from target University in the seven villages from July 2010 to June 2011[4-8].

- Step 1. Health Lectures
  After the questionnaire-based survey at baseline, ten sessions of health lectures were delivered to all the elderly in each of the seven villages by doctors from target University. The health lectures covered various types of knowledge about hypertension, such as pathogenesis, risk factors, prognosis, normal values of blood pressure, fluctuation range of blood pressure, and healthy food.
- Step 2. Personal consultations and distribution of health pamphlets
  There were three kinds of free health pamphlets in this study, i.e. picture-story book, cartoon, and picture poster. Research assistants and undergraduates provided personal consultations and distributed health pamphlets in the elderly people's home. Every elderly person should have one picture poster and either of picture-story book or cartoon.
- Step 3. Peer-education approach
  The peer-education approach was the best method for elderly people, because they had the same background and, therefore, could communicate smoothly. Well-educated elder people were encouraged to organize the seminar, which were participated monthly by elder people in the seven villages. They discussed the topics on hypertension in the seminar. The organizers were financially rewarded and the attendees received gifts from the research team.

- Step 4. Physical examinations
  Physical examinations were conducted for the elderly people in the seven villages. Items included measuring blood pressure, B-ultrasonography, X-rays, and other common physical tests. The researchers kept a health file for every participant.

***Data quality control and statistical analysis***

Each village strictly implemented the HEPH. All questionnaires were checked and the missed items were completed during home visits. To confirm the validity of the obtained data, 5% of the questionnaires were randomly selected and validated by telephone query or home visit. The data was entered by specially trained medical undergraduates from target University. Every elderly people filled out the questionnaire in a separate room in the absence of interference from others. Every completed questionnaire was examined carefully by trained staff. If the questionnaire was filled out falsely or not filled in well and truly, the trained staff contacted the participants to fill in missed information in the questionnaire or to clarify vague answers. All data analysis was performed using the SPSS software, version 13.0.

**Results**

***General demographic characteristics***

Of the 435 elderly people, 45.52% (198) were men and 54.48% (237) were women. The age range was 60-87 years. 29.89% (130) of the participants were illiterate; 45.06% (196) attended primary school and; 19.31% (84) received junior middle school education and 5.75% (25) accomplished senior middle school education. In terms of occupation, 3.91% (17) were managers or executives. 28.28% (123) were workers and 67.82% (295) were farmers. In addition, the survey showed that 25.06% (109) of the elderly people had less than ¥15,000 annual family income, 36.32% (158) were in the range of ¥15,000~¥30,000 and 38.62% (168) earned more than ¥30,000. These data are summarized in Table 1.

***Knowledge level of hypertension among the elderly before the health education***

The knowledge levels of hypertension in the 435 elderly people before the HEPH are presented in Table 2. As shown in this table, a low overall knowledge level of hypertension was noted. Of all the participants, only 31.72% (138 subjects) knew the diagnostic criteria of hypertension. The most frequently incorrectly answered item in Table 2 was how to take medications properly whereas a high percentage of the subjects knew the fact that smoking and drinking can cause blood pressure elevation.

*Table 1. General demographic characteristics*

| Item | Sub-item | Number | percent (%) |
|---|---|---|---|
| Gender | Male | 198 | 45.52 |
| | Female | 237 | 54.48 |
| Age (years) | 60-69 | 254 | 58.39 |
| | 70-79 | 127 | 29.20 |
| | >80 | 54 | 12.41 |
| Education level | Illiterate | 120 | 27.58 |
| | Primary | 222 | 51.04 |
| | Junior high | 74 | 17.01 |
| | Senior high | 19 | 4.37 |
| Occupation | Farmer | 295 | 67.82 |
| | Worker | 123 | 28.28 |
| | Executive | 17 | 3.91 |
| Household income | <¥ 15,000 | 109 | 25.06 |
| | ¥ 15,000-¥ 30,000 | 158 | 36.32 |
| | >¥30,000 | 168 | 38.62 |

*Table 2. Knowledge level of hypertension in the 435 elderly people before the HEPH*

| Item | Numbers with correct answer | Percent (%) | Rank |
|---|---|---|---|
| Smoking can increase blood pressure | 248 | 57.01 | 1 |
| Drinking can increase blood pressure | 226 | 51.95 | 2 |
| High salt diets can increase blood pressure | 195 | 44.83 | 3 |
| Blood pressure have a positive correlation with age | 191 | 43.91 | 4 |
| Exercise can reduce blood pressure | 180 | 41.37 | 5 |
| Agitation and tension can increase blood pressure | 165 | 37.93 | 6 |
| Diagnostic criteria of hypertension | 138 | 31.72 | 7 |
| Obesity increases the risk of hypertension | 134 | 30.81 | 8 |
| Patients should adhere to anti-hypertension agents even when blood pressure declines to a normal level | 131 | 30.11 | 9 |
| Risks of hypertension | 124 | 28.51 | 10 |
| Hypertension is a genetic inheritable disease | 114 | 26.21 | 11 |
| Patients should take medications even without symptoms of hypertension | 81 | 18.62 | 12 |

*Table 3. Needs for medical treatments and health education among 435 elderly people**

| Item | Number | Percent (%) | Rank |
|---|---|---|---|
| To measure blood pressure through family visits at regular intervals | 389 | 89.43 | 1 |
| To offer treatments and medications | 371 | 85.29 | 2 |
| To deliver regular health lectures | 337 | 77.47 | 3 |
| To provide free health education advice/ prescriptions | 295 | 67.82 | 4 |
| To provide medical consultation | 261 | 60.00 | 5 |
| To complete Peer-education approach | 254 | 58.39 | 6 |
| To assist in referral for treatment of a serious disease | 237 | 54.48 | 7 |

** Participants could give more than one response.*

***Needs for medical treatments and health education***

Results about the needs for medical treatments and health education of the 435 elderly people are summarized in Table 3. As demonstrated in this table, measuring blood pressure through family visits at regular intervals was the most requested service 89.43%. Offering treatments and medications were the second most wanted health service 85.29%. In addition, regular health lectures on hypertension prevention and treatment were the third wanted health service 77.47% by these elderly subjects.

***Comparison of hypertension knowledge levels before and after HEPH***

As shown in Table 4, the overall knowledge level of hypertension in the elderly people was significantly improved after implementation of the HEPH activities. Statistical analysis indicated that the percentage of the people who gave correct answers to the questions in Table 4 was significantly improved after the HEPH ($P<0.05$). In addition, the results also showed that the knowledge level of hypertension among elderly females was higher than that of male participants in this study.

**Discussions**

Results from this study indicated that the elderly people in the countryside had a low education level. Only 4.37% of the participants accomplished senior middle school education, whereas a majority of these elderly people (51.04%) only had access to primary school education and 27.58% of them were illiterate. It is generally acknowledged that health literacy plays an important role in health outcomes (9). However elderly people in the countryside of China have a low education level. Hence different health education strategies are needed for the elderly people with various levels of health literacy. For those who received little

*Table 4. Comparison of hypertension knowledge levels before and after the health education (%)*

| Item | Male | | | Female | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | Before- | After- | P-value | Before- | After- | P-value | Before- | After- | P-value |
| Knowing the normal blood pressure in adults | 20.92 | 60.00* | (<0.05) | 19.08 | 61.15* | (<0.05) | 20.00 | 60.58* | (<0.05) |
| Blood pressure should be measured yearly after the age of 35 years | 11.95 | 64.83* | (<0.05) | 10.12 | 66.21* | (<0.05) | 10.03 | 65.52* | (<0.05) |
| Hypertension can cause stroke | 20.22 | 61.38* | (<0.05) | 18.39 | 60.00* | (<0.05) | 19.30 | 60.96* | (<0.05) |
| Hypertension can cause coronary heart diseases | 28.96 | 45.98* | (<0.05) | 27.58 | 46.21* | (<0.05) | 28.27 | 46.10* | (<0.05) |
| Elderly patients with hypertension should take medications as prescribed. | 35.86 | 85.97* | (<0.05) | 33.79 | 86.90* | (<0.05) | 34.83 | 86.44* | (<0.05) |
| Elderly patients with hypertension should take low salt and low fat diets. | 42.99 | 86.44* | (<0.05) | 39.08 | 87.36* | (<0.05) | 41.03 | 86.90* | (<0.05) |
| Elderly patients with hypertension should do certain amount of exercises. | 24.83 | 56.32* | (<0.05) | 20.69 | 56.55* | (<0.05) | 22.79 | 54.44* | (<0.05) |
| Elderly patients with hypertension should control their body weight. | 37.93 | 57.01* | (<0.05) | 37.24 | 59.31* | (<0.05) | 37.59 | 58.16* | (<0.05) |
| Elderly patients with hypertension should avoid agitation and tension. | 19.08 | 58.39* | (<0.05) | 21.15 | 61.60* | (<0.05) | 20.11 | 59.00* | (<0.05) |
| Obesity is more likely to cause hypertension | 25.29 | 72.41* | (<0.05) | 22.76 | 82.30* | (<0.05) | 24.03 | 77.35* | (<0.05) |
| Family history of hypertension will increase the risk of hypertension. | 11.04 | 64.38* | (<0.05) | 10.35 | 65.06* | (<0.05) | 10.70 | 64.72* | (<0.05) |
| Heavy smokers are more likely to develop hypertension. | 46.21 | 72.87* | (<0.05) | 47.36 | 80.46* | (<0.05) | 46.79 | 76.67* | (<0.05) |
| People with A-type psychological characteristic or personality are more likely to develop hypertension. | 17.93 | 65.28* | (<0.05) | 17.01 | 67.59* | (<0.05) | 17.47 | 66.44* | (<0.05) |
| People with high level of triglyceride or cholesterol are more likely to develop hypertension. | 22.30 | 85.97* | (<0.05) | 20.92 | 85.06* | (<0.05) | 21.61 | 85.52* | (<0.05) |
| People with high salt diets are more likely to develop hypertension. | 58.85 | 96.09* | (<0.05) | 60.22 | 98.39* | (<0.05) | 59.54 | 97.24* | (<0.05) |

** P<0.05*

education, health knowledge should be conveyed using simple explanations and acceptable technique, such as a peer-education approach. As for those with higher health literacy, offering cartoon health pamphlets and journals may be more appropriate. In addition, owing to physical aging, the elderly people often show slow response and poor memory. Therefore special health education regimens for this group are needed in order to achieve a better effect for disease prevention and treatment. In the present study, we displayed that the overall knowledge level about hypertension was low before the HEPH. In particular, lack of knowledge regarding the proper use of anti-hypertension agents and the potential harm of hypertension was observed. Furthermore, our study showed that most of the elderly people expressed their needs for taking blood pressure as well as medical treat-

ments and health education. The results obtained in this research indicated that they had a high demand for knowing their health conditions, especially for knowledge of hypertension. Therefore we established health records for all participants and measured their blood pressure at regular intervals during the course of study. Moreover, in an attempt to help them remove negative living habits and prevent the occurrence of hypertension and its complications, we also carried out extensive health education, including regular health lectures, peer-education approach, distribution of cartoon health pamphlets and the like. Indeed, a marked difference of hypertension knowledge levels before and after the health education revealed that health education might help reduce the risk of hypertension in aged people, thereby enhancing their quality of life in the countryside.

Many elderly people living in the countryside fail to get proper medical treatments due to their low education levels, a lack of knowledge about hypertension and low economic status. Hence more attention should be directed to these elderly people in the countryside. Medical and social workers should work together to help improving health literacy among the elderly through special methods of health education in the countryside.

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**Reference**

1. *Yao WX, Wang XQ, Li XM, et al. Health education of hypertension for the elderly in Gengxi countryside of Zhouzhi County of Shaanxi province. Northwest Medical Education 2006; 14: 307-8. (in Chinese)*

2. *Li ZR. Health education for in-patients with hypertension in Fenyang hospital, Shanxi province. Chinese Journal of Health Education 2001; 6: 3771. (in Chinese)*

3. *Williams MV, Parker RM, Baker DW, et al. Inadequate functional health literacy among patients at two public hospitals. JAMA 1995; 274: 1677-82.*

4. *Yang YY. Effects of community nursing on aged people with hypertension. Nursing and Rehabilitation Journal 2004; 3: 2711. (in Chinese)*

5. *Jiang J, Bao KZ. KAP About High Blood Pressure Preventing and Controlling in Ningbo City Zone. Practical Preventive Medicine 2005; 6: 564-6. (in Chinese)*

6. *Williams MV, Baker DW, Parker RM, et al. Relationship of functional health literacy to patients. Knowledge of their chronic disease. Arch InternMed 1998; 158:166-72.*

7. *Cheng QY, Li JF. Effects of health education for community residents with hypertension. Chinese Journal of Public Health 2005; 7: 879-80. (in Chinese)*

8. *Baker DW, Wolf MS, Feinglass J, et al. Health Literacy and Mortality Among Elderly Persons. Arch Intern Med 2007; 167(14): 1503-9.*

9. *Berkman ND, Sheridan SL, Donahue KE, et al. Low health literacy and health outcomes: an updated systematic review. Ann Intern Med 2011; 155(2): 97-107.*

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# A case of serious neurological defect due to repeated spinal epidural blood patch

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**Abstract**

A spinal epidural patch using autologous venous blood injected into the epidural space is a procedure carried out in the treatment of persistent cerebrospinal fluid leakages that may develop after such interventions as lumbar catheter placements and lumbar punctures, which occasionally result in complications. Reported complications from this procedure include lumbar pain and radiculopathy, however no serious neurological problems occurring after this procedure have been reported up to date. This paper offers a description of the emergency decompression surgery performed to treat a case of cauda equina syndrome that developed after repetitive spinal epidural blood patch applications to treat a cerebrospinal fluid leakage following a lumbar puncture for spinal anesthesia.

**Key words:** CSF fistula, epidural patch, spinal epidural hematoma.

## Introduction

The injection of autologous venous blood into the epidural space to prevent spinal cerebrospinal fluid leakage is a form of iatrogenic spinal epidural hematoma known as an epidural patch, and is an intervention performed to prevent persistent spinal CSF leakage with occasionally reported complications. (1) A case of cauda equina syndrome, which developed after repetitive spinal epidural blood patch applications to treat a cerebrospinal fluid leakage after a lumbar puncture performed for spinal anesthesia is reported.

## Case

A 17-year-old female patient presented with a headache two days after surgery with spinal anesthesia. The patient was thought to be suffering from cranial hypotension due to a CSF leak after a lumbar puncture between the second and third lumbar vertebrate. A spinal epidural patch was applied with an injection of 2 ml of autologous venous blood at the level of L3-L4. The headache eased over the following days, but recurred three days after the procedure, and so further spinal epidural patch applications of 2 ml of autologous venous blood were made on different days at the same site. Paraplegia, parahypoaesthesia and urinary retention developed a hours after the final patch application. A spinal epidural hematoma in the left posterolateral area of the lumbar region, extending from L2 to L5, was identified from the MRI (Figure 1 and 2). Spinal epidural hematoma was removed through a left L3, L4 and L5 hemilaminectomy. The neurological status of the patient improved after surgery, and a control MRI indicated a complete disappearance of the hematoma (Figure 3). The patient was discharged on the seventh day after surgery following a neurological examination, during which no neurological deficits were found.

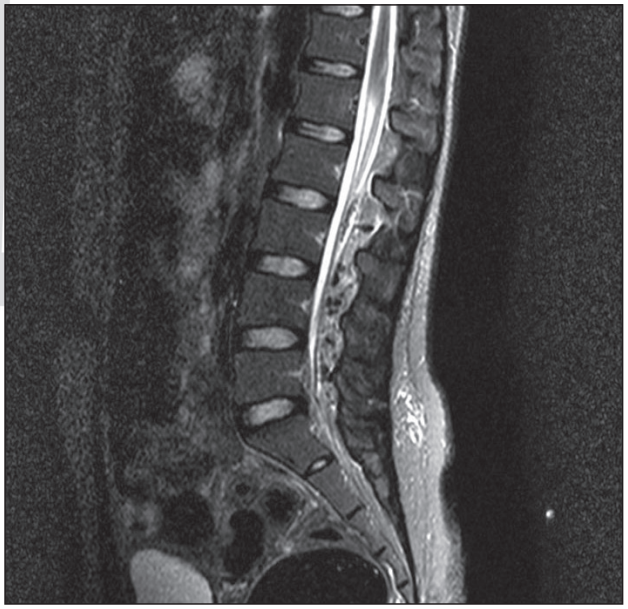

*Figure 1. Preoperative sagittal MRI showing spinal epidural hematoma at lombar dorsal spinal epidural area*

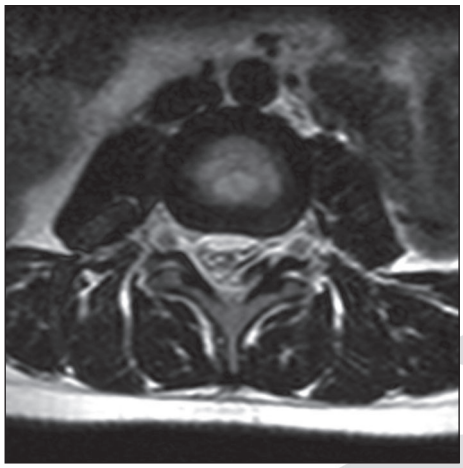

*Figure 2. Preoperative axial MRI showing hematoma compressing lomber dural sleeve*

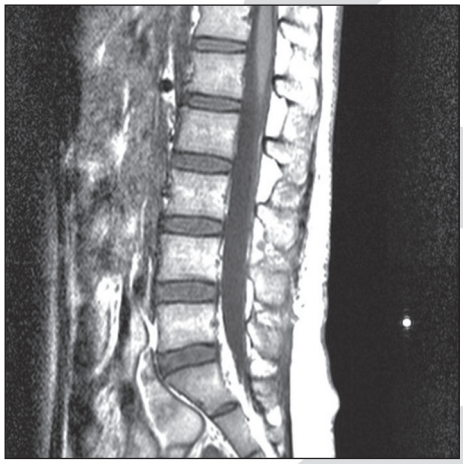

*Figure 3. Postoperative sagittal MRI with no pathological findings*

## Discussion

The causes of spinal epidural hematoma is hematological disorders, treatments using anticoagulants, vascular malformations, hypertension, epidural tumors and trauma. In addition, spinal surgery, lumbar puncture and spinal epidural patches with autologous venous blood are among the iatrogenic causes of spinal epidural hematoma. No etiological factors can be found in 40% of cases of spinal epidural hematoma, which defined as spontaneous epidural hematoma (2).

Patch applications using injections of epidural autologous venous blood into the area in which leakage is suspected for the treatment of CSF leaks that develop after lumbar punctures for CSF sampling and spinal anesthesia, and for fistulas that develop after surgical procedures, have been used for many years with very few reported complications. Among the reported complications are lower back pains, radiculopathy, and spinal subdural or epidural hematoma. No advanced level neurological deficits resulting from patch application have been reported to date (1,4).

Although complications after spinal epidural patch applications are uncommon and are rarely serious enough to cause advanced neurological deficits, according to literature serious problems are possible, as with the case under discussion. Reasonable preventive measures to decrease complications include patch applications only by experienced hands, and not more than once; and the patient should be closely monitored after the intervention with serial neurological examinations and MRIs when a neurological deficit occurs, which will aid in the prevention of irreversible complications. In situations of severe neurological problems due to a hematoma, as was the situation in the case under discussion, the treatment of choice is the evacuation of epidural blood through emergency surgery.

## References

1. *Desai MJ, Dave AP, Martin MB. Delayed radiculer pain following two large volume epidural blood-patch for post lumbar puncture headache: A case report. Pain Physician. 2010; 13: 257-62.*

2. *Göksel HM, Karadağ Ö, Gürelik M, Özüm Ü. Spontan Spinal Epidural Hematom: Olgu Sunumu. Türk Nöroşirürji Dergisi. 2000; 10: 218-20.*

3. *Lo CC, Chen JY, Lo YK, Lai PH, Lin YT. Spontaneous spinal epidural hematoma: a case report and review of the literatures. Acta Neurol Taiwan. 2012; 21: 31-4.*

4. *Tekkök IH, Carter DA, Brinker R. Spinal Subdural Haematoma as a Complication of Immediate Epidural Blood-Patch. Can J Anaesth. 1996; 43: 306-9.*

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# Endoscopic diagnosis, screening & surveillance and treatment of Barrett's esophagus

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**Abstract**

Barrett's esophagus (BE) is an acquired condition in which the squamous epithelial lining of the lower esophagus is replaced by a columnar epithelium due to chronic gastroesophageal reflux. The prevalence of BE has ranged from 0.9% to 4.5%. The rate of progression from BE to esophageal adenocarcinoma is 0.5% per patient-year. Human studies show that the reflux of bile parallels, acid reflux and increases with the severity of gastroesophageal reflux disease (GERD), being most marked in BE. However, recent ex vivo studies suggest that pulses of acid reflux may be more important than bile salts in the development of dysplasia or adenocarcinoma in Barrett's epithelium. The diagnosis of BE can be suspected when, during endoscopic examination, columnar epithelium is observed to extend above the gastroesophageal junction (GEJ) into the tubular esophagus. To identify relevant articles for this review, a PubMed search was conducted using relevant key words and phrases. In addition, reference lists from identified manuscripts were searched and bibliographies of relevant studies were also reviewed. Although, guidelines for the diagnosis, surveillance and management of BE were published, the main goal in the management of premalignant condition would be the permanent elimination of Barrett's mucosa. Current therapeutic options are limited or still in the investigational stages. This review summarizes the endoscopic diagnosis, screening, surveillance and introduces endoscopic ablative modalities currently used.

**Key words:** Argon plasma coagulation; Barrett's esophagus; Endoscopic mucosal resection; Gastroesophageal reflux disease; Multipolar electrocoagulation.

## Introduction

Barrett's esophagus (BE) is the condition in which any extent of metaplastic columnar epithelium that predisposes to cancer development replaces the stratified squamous epithelium that normally lines the distal esophagus (1), which gives a red appearance to the esophagus on endoscopic examination. Many etiological factors are involved in the development of Barrett's esophagus (BE), such as gastroesophageal reflux disease (GERD), with inflammatory stress leading to metaplastic transformation of the mucosa. Advanced age, male sex, white race, symptoms of reflux and obesity are the risk factors for Barrett's esophagus.

Upper endoscopy is required for diagnosis which is based on initial endoscopic investigation and histopathologic examination of specimens that obtained from biopsy of the endoscopically visible Barrett's segment. Those affected segment may extend to variable lengths. BE that more commonly seen among Asian populations have short-segment (< 3 cm), while in Western populations having long segment (> 3 cm) type seen mostly. Societal guidelines generally have recommended endoscopic surveillance for patients with BE at intervals of three to five years have been suggested for patients who have no dysplasia, six months to one year for those found to have low-grade dysplasia (LGD), and every three months for patients with high-grade dysplasia (HGD) who receive no ablation therapy (2). Screening a high-risk group such as men with GERD will result in the detection of more patients with BE, many of whom are asymptomatic. BE occurs more often in men than women. The male to female ratio is approximately 2: 1 (3).

The aim of this literature review is to summarize the endoscopic diagnosis, screening, surveillance and introduces endoscopic ablative modalities currently used.

### *Prevalance of BE*

The prevalence of BE in the general U.S. population is not known, while the prevalence of GERD is increasing both in the West and in Asia and this may justify strategies for prevention and early de-

tection of BE. Hiatal hernia is also associated with the presence of BE (4). In one of the population-based study conducted in Sweden, BE was diagnosed in 1.6% of 3000 study participants (5). Studies have reported inverse associations between the presence of BE and consumption of red wine, Helicobacter pylori (HP) infection, and black race (6). Esophageal adenocarcinoma has been estimated to develop in about 0.5%–1.0% of patients with BE annually (7) and is now the most rapidly increasing cancer in the Western world**.** The incidence of adenocarcinoma in patients with BE increases with the degree of dysplasia from approximately 0.5% per year (8) in nondysplastic BE to as high as 15% to 20% per year in subjects with high-grade dysplasia (HGD) (9,10). BE itself does not cause symptoms. The extent of dysplasia is usually characterized by pathologists as mild or low grade dysplasia (LGD), and severe or high-grade dysplasia (HGD). Current techniques for endoscopic monitoring of the disease represent a large cost burden, the value of which has been called into question (11).

***Diagnosis***

Individuals who have experienced acid reflux symptoms for a number of years should undergo an upper endoscopy exam to determine if they have BE. BE is marked by the presence of columnar epithelia in the lower portion of esophagus, replacing the normal squamous cell epithelium that is metaplasia. This metaplasia confers an increased risk of adenocarcinoma (12). The line at which the columnar epithelium transitions to the squamous epithelium (i.e., the squamocolumnar junction) is known as the Z-line. Normally, the Z-line corresponds to the gastroesophageal junction (GEJ). In BE, the Z-line is displaced proximally relative to the gastroesophageal junction.

BE is diagnosed by endoscopy and histology. An endoscopy procedure is performing to look at the lining of the esophagus and biopsies to examine samples of suspect tissue. The biopsy is examined in a lab to see whether the normal squamous cells have been replaced with Barrett's cells (13). Long-segment BE measures 3 cm or more from the gastroesophageal junction, while short-segment BE is less than 3 cm from the gastroesophageal junction (5). More metaplasia would predispose to more cancer, and one study (14) has shown this to be the case. On the pathologic examination, intestinal metaplasia can be identified by the presence of goblet cells on biopsy of the esophageal mucosa. There are may be of two types of metaplastic columnar cells: gastric that similar to those in the stomach, which is not technically BE or colonic that is similar to cells in the intestines. Colonic-type metaplasia is the type of metaplasia associated with risk of malignancy in genetically susceptible people. Histochemical stain Alcian blue (pH 2.5) is used to distinguish true intestinal-type mucins from their histologic mimics. Recently, immunohistochemical analysis with antibodies to CDX-2 (specific for mid and hindgut intestinal derivation) has also been utilized to identify true intestinal-type metaplastic cells. It has been shown that the protein AGR2 is elevated in BE (15) and it can be used as a biomarker for distinguishing BE from normal esophageal epithelium (16). After the initial diagnosis of BE are rendered, affected people undergo annual surveillance to detect changes that indicate higher risk to progression to dysplasia.

***Screening and surveillance for BE***

Screening and surveillance for BE remain very controversial topics. Screening refers to the initial endoscopic examination performed in subjects for the detection of BE and /or esophageal adenocarcinoma while surveillance is the continued observation of patients with BE by serial endoscopy for the detection of dysplasia and/or early cancer. There are lots of challenges to screening for BE such as: inability to predict who has BE prior to endoscopy, the lack of evidence based criteria, the invasiveness and expense of endoscopy, and the increasing documentation of a subgroup of patients with BE who lack reflux symptoms. Predictors included age >40 (17), heartburn (17,18), long duration GERD symptoms (≥13 years), and male gender (18). Histological confirmation of disease varies with the length of columnar appearing mucosa identified at endoscopy, with suspected short-segment disease confirmed in only about 25% of cases and long-segment disease confirmed in 44%–80% of cases (19). More than 20% of patients without confirmation of intestinal metaplasia at initial endoscopy have it at later endoscopy probably because of sampling error or interim development of intestinal metaplasia after the first examination (20).

A retrospective study of endoscopic and pathology reports from 15 hospitals in the Netherland revealed that adherence to the Seattle protocol was 79% for cases in which Barrett's metaplasia involved only up to 5 cm of the distal esophagus but diminished with increasing extent of metaplasia to the point that there was only 30% adherence among cases with metaplasia involving 10-15 cm of the esophagus (21). Esophageal biopsy specimens taken for evaluation of BE and who had endoscopy reports that documented the extent of esophageal columnar lining (22) was found in only 51% of cases. As in the previous study, adherence to the protocol varied inversely with the extent of Barrett's metaplasia.

Streitz et al. (23) studied 77 patients treated for esophageal adenocarcinoma. Among them 19 patients, the cancers were found during surveillance endoscopies performed, while 58 patients with symptoms of esophageal cancer, and BE was first diagnosed when their tumors were resected. Fountoulakis et al. (24) studied a cohort of consecutive patients those gone for esophagectomy for HGD or adenocarcinoma in BE, showed, with 1- and 3- year survival rates for the surveillance and no-surveillance groups of 88% versus 67% and 80% versus 31%, respectively. Corley et al. (25) studied for 589 patients with adenocarcinoma of the esophagus or gastric cardia those diagnosed during 1990 to 1998. Only 23 patients who were known to have had BE for at least 6 months before the cancer was diagnosed and 15 had their tumors discovered during surveillance endoscopy.

A more recent analysis, Cooper et al. (26) included 2754 patients with a new diagnosis of esophageal adenocarcinoma, having an endoscopy performed 3 years to 6 months before the diagnosis of cancer was associated with an improvement in median survival from 7 months (for patients with no prior endoscopy) to 11 months. In a case-control study, Kearney et al. (27) found that a group of 245 patients who had GERD and adenocarcinoma of the esophagus or gastric cardia were significantly less likely to have undergone endoscopy in the 1 to 8 years before the index date than 980 control subjects (matched by age, sex, and race) who had GERD without cancer (adjusted odds ratio, 0.66; 95% CI, 0.45– 0.96).

The American College of Gastroenterology (ACG) guidelines state that "patients with chronic GERD symptoms are those who are most likely to have BE and should undergo upper endoscopy" (28). If BE is detected, as the part of surveillance for precancerous cell changes called dysplasia, should be done an endoscopic biopsy every two to three years to patients. If intraepithelial neoplasia is found in the Barrett's epithelium, appropriate intervention, with follow-up surveillance at appropriate time-intervals is generally recommended (12). It is recommended that 4-quadrant biopsy samplings of the Barrett's epithelium are taken at 1- to 2-cm intervals along the entire segment of affected esophageal tissue. Surveillance procedures call for periodic examinations, with the frequency dictated by the degree of dysplasia observed (7, 1). Patients with BE with dysplasia would undergo surveillance endoscopy every 6 months for low-grade dysplasia or every 3 months for high-grade dysplasia as long as dysplasia was noted at least once during the preceding 12 months (29). It has been suggested that screening should focus on patients with GERD who have risk factors for BE, such as male sex, white race, an age of more than 50 years, and a long history of symptoms (more than five years).

Endoscopic surveillance programs have been established in an effort to diagnose cancer at an early stage in patients with BE. The numbers of published case–control and cohort studies have shown that endoscopic surveillance is significantly associated with both an earlier stage of esophageal adenocarcinoma at diagnosis and improved survival (30). On the survey in UK by Mandal et al. only 76% of respondents considered that surveillance was worthwhile. In those who considered surveillance worthwhile, 83% used sub-selection based on age, length of Barrett's or presence of ulcer or stricture (30). Although**,** no randomized, controlled trials have evaluated the efficacy of surveillance, and also not clear whether surveillance reduces the mortality from esophageal cancer**,** most of the major gastroenterological societies and published guidelines recommend surveillance of patients with BE. There are several factors which expected benefits of current surveillance strategies such as the low overall incidence of cancer in BE patients, the absence of a previous diagnosis of BE in the majority of patients with esophageal adenocarcinoma, and difficulties in the diagnosis of dysplasia which is a high missing rate on evaluation of random bi-

opsy specimens and high variation among pathologists in the interpretation of biopsy findings (31,32).

Recent studies suggest a risk of cancer is 0.5% or less annually. Sharma et al. (33) studied the incidence of cancer was 1 case in 212 patient-years of follow-up (0.5% per year). One of the meta-analysis (34) shows the incidence rates for esophageal adenocarcinoma range from 0.6% to 1.6% per year in patients with LGD. Esophageal adenocarcinoma development rate is high among patients with high-grade dysplasia, with an estimated incidence of 6.6 cases per 100 patient-years (95% confidence interval, 4.9 to 8.2) in a recent meta-analysis (35). In a randomised crossover study (36) of 121 patients undergoing endoscopy for screening and surveillance of BE, the prevalence of disease was similar between conventional endoscopy (26%) and unsedated small-calibre endoscopy (30%). In a single-centre prospective study (37) of 90 patients undergoing screening or surveillance for BE, capsule endoscopy was 67% sensitive and 84% specific for identification, diagnosing 14 of 21 cases who were confirmed by biopsy. Somewhat improved operating characteristics of capsule endoscopy were seen in 89 patients by French investigators (38). Recently, enhanced optical imaging techniques i.e., High-resolution, white-light Endoscopy, Magnification endoscopy, Chromoendoscopy, Narrow-band imaging (electronic Chromoendoscopy), Autofluorescence imaging, Confocal microscopy have been suggested to improve the efficiency and accuracy of endoscopic surveillance. Most of these techniques have not been directly compared with standard endoscopy, but use of narrow-band imaging and confocal laser endomicroscopy suggest a high rate of accuracy (85 to 92%) in the diagnosis of neoplasia in patients with BE (39, 40). The cost-effectiveness of endoscopic screening and surveillance is still unknown, and it depends upon poorly described factors such as the accuracy of the test to diagnose BE, the risk of cancer in patients with the condition, and the cost of endoscopy and histological analysis (41). One analysis suggested that, although one screening endoscopy was highly cost-effective, subsequent surveillance endoscopies in patients whose biopsies showed only BE with no dysplasia were very cost-ineffective, adding only a few extra days of life expectancy at an extra cost of thousands of US dollars (42). In a prospective double-blind study (43) of 33 patients, the technique had an accuracy of 78% for the detection of dysplasia in patients with BE. In a study of 63 patients with BE and associated neoplasia, changes with 98.1% and 92.9% sensitivity and 94.1% and 98.4% specificity, respectively (96.8% and 97.4% accuracy, respectively) (40).

***Endoscopic treatment of BE***

Endoscopic therapies can be further subdivided into tissue-acquiring and non-tissue-acquiring modalities.

***Multipolar electrocoagulation (MPEC)***

MPEC indicates that this is a safe, effective method to ablate BE over the long term in combination with acid suppression, suggested findings from a 10-year follow-up study. Allison et al. (44) reported 166 patients were recruited for the study; 139 completed at least 10 years of follow-up. Complications developed in less than 5% of patients. Recurrent BE occurred in less than 5% of patients. No adenocarcinoma or high grade dysplasia of the esophagus developed in any of the patients. Sharma et al. (45) compared Argon Plasma Coagulation (APC) and MPEC for ablation of nondysplastic BE in 35 patients. The complete remission rate of Barrett's epithelium was comparable in both groups (75% vs 63%; $P$=0.49). Subsequently, Sampliner et al. (46) documented successful removal of BE 45 of 58 patients in a multicenter study.

***APC***

It is considered as a standard technique for ablation especially of short segments of BE. In most studies APC was used to ablate nondysplastic BE. Complications of APC are strictures, relatively rarely bleedings and very rarely perforations. Van Laethem et al. (47) studied APC is safe and effective in the management of high grade dysplasia and in situ adenocarcinoma associated with BE. Complete eradication of high grade dysplasia and in situ adenocarcinoma was achieved after a mean number of 3.3±1.5 APC sessions in 8 of 10 patients (80%). Attwood et al. (48) treated 29 patients with APC with a mean follow-up period of 37 months. Twenty-five patients had elimination of high grade dysplasia and 22 patients had elimination of BE.

APC appears to be more effective than Photodynamic Therapy (PDT). A total of 68 patients with BE were randomized to APC ($n$ = 34) or PDT ($n$=34). In the APC group with 33 of 34 (97%)

ablated, compared with 17 of 34 (50%) in the PDT group (49). Madisch et al. (50) studied 70 patients with histologically proven nonneoplastic BE, complete BE ablation was achieved by APC and 120 mg omeprazole daily. Among them 66 patients (94.4%) underwent further surveillance endoscopy. In none of the patients, intraepithelial neoplasia nor an esophageal adenocarcinoma was detected.

***Radiofrequency Ablation (RFA)***

RFA seems to be the method for ablation with the highest rates of Barrett's eradication with a very low complication rate and an almost absent risk of residual 'buried glands (51, 52). A focal device (HALO-90) fits over the tip of the endoscope and can be used for ablation of smaller areas (53). In a dose-response and efficacy study in patients with nondysplastic BE who underwent treatment with the HALO360 system, 70% of 70 patients had complete ablation of BE after 1 year of follow-up (53). Two recently published (51, 52) studies from the Amsterdam group combined ER of visible neoplastic lesions with circumferential and focal RFA of the remaining BE containing high grade dysplasia in 23 patients. Ablation without prior endoscopic resection was performed in 10 patients with flat high-grade intraepithelial neoplasia (HGIN). Complete elimination of neoplasia and Barrett's metaplasia was possible in all of the 23 included patients, and none of the 836 biopsies of the neosquamous mucosa contained subsquamous BE.

In a US multicenter registry with 16 centers (54), there 142 patients with high-grade dysplasia underwent a mean of 1 ablation session with the HALO360 system. A complete removal of high-grade intraepithelial neoplasia was confirmed in 90.2% and of Barrett's epithelium in 54.3% (54). It also seems to be the ideal addendum to endoscopic resection for ablation of the remaining nondysplastic Barrett's epithelium after successful resection of all localizable high-grade intraepithelial neoplasia and adenocarcinoma. If there is evidence of fibrosis in the wall of the esophagus, an ablation balloon with a diameter smaller than that determined by the measuring balloon is used to prevent esophageal tears; RFA is not recommended for patients with esophageal strictures because inflation of the treatment balloons (typical diameters of 28 to 31 mm) could cause perforations. During a follow-up period of 30 months, by using a combination of the balloon-based and focal RFA devices, an uncontrolled study has described complete eradication of Barrett's epithelium in 60 of 61 patients (55). From 19 US centers, a multicenter, randomized, sham-controlled trial (9) investigated the efficacy of RFA in 127 patients with low-grade intraepithelial neoplasia ($n$=63) and high-grade intraepithelial neoplasia ($n$=64). Patients were randomized (2: 1) to RFA or sham treatment. The 1-year interim analysis was able to show that 74% of patients randomized to RFA achieved complete clearance of Barrett's metaplasia compared with 0% in the sham arm. RFA successfully removed high-grade intraepithelial neoplasia in 83% of patients (10 of 12) and low-grade intraepithelial neoplasia in 100% (23 of 23). In the sham group, 0% of patients had clearance of high-grade intraepithelial neoplasia and 36% of low-grade intraepithelial neoplasia.

***Cryotherapy***

The latest technique of ablation therapy applied to BE with the least experience in human beings is cryotherapy. Cryotherapy works by freezing the mucosa to induce cell death. Two systems have been used including one sprays liquid nitrogen through an open-tip catheter (56) the other forces a cryogenic refrigerant (carbon dioxide) through a catheter that also provides venting (57). There are 2 different devices for cryotherapy in the gastrointestinal tract.

Raju et al. (58) have reported esophageal cryotherapy could result in a dose-dependent injury to the esophagus. In a single-center, retrospective study, among 36 patients, 33 patients (92%) achieved a complete response. Remaining of three low-grade dysplasia (1 patient) and intestinal metaplasia (2 patients) was diagnosed (59). Nonrandomized and uncontrolled studies show success rates comparable to other ablative modalities for the treatment of BE with HGD, with complete eradication of dysplasia seen in 87-96% and complete eradication of intestinal metaplasia in 57-96% of treated patients. In early-stage esophageal cancer, spray cryotherapy appears to have a unique role, eliminating mucosal cancer in 75% of patients, including those who have failed other modalities (60).

***Photodynamic therapy (PDT)***

PDT is an established endoscopic technique for ablating BE with high-grade dysplasia or early-

stage intraepithelial neoplasia and has been successfully used to treat early neoplasia in BE for more than one decade**.** The most common clinically significant adverse effect seen in > 30% of the patients who were treated with PDT developed esophageal strictures (10), which is usually superficial and might be dilated effectively with standard endoscopic accessories, such as endoscope balloon or Savary dilators (6). In BE, it has involved a variety of different agents, including porfimer sodium and 5-aminolevulinic acid (5-ALA) (10, 62). In a large multicentric randomized trial utilizing intravenous porfimer sodium, a photosensitizer, 255 patients with high-grade intraepithelial neoplasia were treated by PDT and proton pump inhibitors (PPIs) or PPI only. Ablation of all high-grade dysplasia was noted in 77% of patients in the PDT arm versus 39% in the control arm. Significantly fewer patients progressed to cancer in the PDT arm than in the PPI arm (13 vs. 28%) (10). However, complications occurred in 94% of the PDT group. Results were similar after 5 years of follow-up (62).

PDT is a highly effective, safe and minimally invasive first-line treatment for patients with Barrett's dysplasia and mucosal adenocarcinoma. Wolfson et al. (63) used porfimer sodium photodynamic therapy to treat patients with BE and high-grade dysplasia or mucosal carcinoma. Among 102 patients with Barrett's HGD (69 patients) or mucosal adenocarcinoma (33 patients) have been treated with PDT using porfimer sodium as an alternative to oesophagectomy. Overall treatment results found complete ablation of glandular epithelium with one course of PDT in most patients (56%). Prasad et al. (64) demonstrated in 126 patients that p16 allelic loss predicted decreased response to PDT with an odds ratio of 0.32. Other independent predictors of loss of dysplasia were the length of the Barrett's segment and the performance of PDT.

Porfimer-photodynamic therapy with supplemental Nd: YAG photoablation and continuous treatment with omeprazole reduces the length of Barrett's mucosa, eliminates HGD, and, by comparison with historical data, may reduce the expected frequency of carcinoma. Overholt et al. [65] performed Porfimer-photodynamic therapy in 103 patients. The Nd: YAG laser was used to photoablate small areas of residual or untreated Barrett's mucosa. Acid suppression was maintained in all patients (omeprazole, 20 mg twice a day). Among 103 patients, intention-to-treat success rates were 92.9%, 77.5%, and 44.4% for, respectively, LGD, HGD, and early stage carcinoma groups. In addition, PDT is associated with up to a 36% rate of strictures and causes temporary cutaneous photosensitivity, but, in long-term follow-up, the strictures all responded to endoscopic therapy (10,62). PDT is well tolerated and highly effective with long-term complete remission rates of more than 94% (66). PDT with porfimer sodium is expensive and associated with a high complication rate. Photosensitivity and stricture formation in up to 30% of patients are important drawbacks of this method.

***Endoscopic Mucosal Resection (EMR)***

There are two concepts for EMR: lesions can either be removed en bloc or piecemeal. EMR allows for more precise characterization of neoplasia, less interobserver variation among pathologists, and more accurate assessments of dysplasia grades and the depths of invasion (67). Endoscopic therapy might be curative for most patients with neoplasms confined to the mucosa. For tumors that involve the submucosa, however, the rate of metastasis to the lymph nodes exceeds 20% (68). Until recently, few studies with large patient numbers or long follow-up were available for EMR. Recently, Pech O et al. (66) published endoscopic treatment of early Barrett's neoplasia in 349 patients. EMR was performed in 279 patients, PDT in 55 patients and both methods were combined in 13 patients; two patients received APC. It was highly effective treatment with a remission rate of 96.6%. However, metachronous and recurrent neoplasia was observed in 21.5% of cases during a follow-up of more than 5 years. Then most patients were retreated successfully and long-term complete response was achieved in 84%.

A recent study (69), 100 consecutive patients were treated with EMR and followed up for a mean of 3 years with a calculated 5-year survival rate of 98%. Sixty-nine percent of the patients had short-segment BE. The recurrent or metachronous cancer rate was 11% with successful re-treatment with EMR. The Amsterdam group (70) treated 37 patients with stepwise radical endoscopic concept. Complete eradication of early neoplasia was achieved in all 37 patients treated in a median number of three sessions, and complete removal

of all Barrett's mucosa was achieved in 33 patients (89%). No recurrences had been observed after 11 months. There is drawback of EMR with the suck-and-cut technique appears to be that only small lesions with a diameter of less than 20mm can be resected en bloc with tumour-free lateral margins.

## Conclusion

BE is a premalignant metaplastic process that typically involves the distal esophagus. Its presence is suspected by endoscopic evaluation of the esophagus, but the diagnosis is confirmed by histologic analysis of endoscopically biopsied tissue. Screening and surveillance for BE remain very controversial topics. Identified guidelines for both screening and surveillance have been proposed, they are variable and not evidence-based. The rapid advance of endoscopic therapeutic techniques in the last decade has made it an acceptable alternative to an esophagectomy in patients with high-grade dysplasia and early esophageal adenocarcinoma (EAC). Patients diagnosed with BE are recommended to undergo endoscopic surveillance of BE every 3–5 years. Endoscopic treatment is carried out effectively and safely if patients fulfill certain low risk criteria for lymph node involvement. New endoscopic therapies have evolved that provide an effective, nonsurgical means of eradicating BE with dysplasia and early EAC. Although there have not been any randomized controlled trials comparing endoscopic therapies with esophagectomy in patients with high-grade dysplasia and early esophageal cancer, limited long-term outcome has been promising and shows a high rate of eradication of dysplasia and intestinal metaplasia with endotherapy. Endoscopic treatments have the additional advantages of being outpatient procedures with shorter recovery times.

A combination of different endoscopic treatments may provide the best outcomes. Given that relatively few patients need these treatments each year, offering them at specialized centres will concentrate clinical expertise and be the most cost-effective approach.

## References


1. *American Gastroenterological Association Medical Position Statement on the Management of Barrett's Esophagus. Gastroenterology 2011; 140: 1084–91.*
2. *Wang KK, Sampliner RE. Updated guidelines 2008 for the diagnosis, surveillance and therapy of Barrett's esophagus. Am J Gastroenterol 2008; 103: 788-97.*
3. *Cook MB, Wild CP, Forman D. A systematic review and meta-analysis of the sex ratio for Barrett's esophagus, erosive reflux disease, and nonerosive reflux disease. Am J Epidemiol 2005; 162: 1050-61.*
4. *Avidan B, Sonnenberg A, Schnell TG, Sontaq SJ. Hiatal hernia and acid reflux frequency predict presence and length of Barrett's esophagus. Dig Dis Sci 2002; 47: 256–64.*
5. *Ronkainen J, Aro P, Storskrubb T, et al. Prevalence of Barrett's esophagus in the general population: an endoscopic study. Gastroenterology 2005; 129: 1825-31.*
6. *Kubo A, Levin TR, Block G, et al. Alcohol types and sociodemographic characteristics as risk factors for Barrett's esophagus. Gastroenterology 2009; 136: 806-15.*
7. *Spechler SJ. Clinical practice. Barrett's Esophagus. N Engl J Med. 2002; 346: 836–42.*
8. *Shaheen NJ, Richter JE. Barrett's oesophagus. Lancet 2009; 373: 850–61.*
9. *Shaheen NJ, Sharma P, Overholt BF, et al. Radiofrequency ablation in Barrett's esophagus with dysplasia. N Engl J Med 2009; 360: 2277–88.*
10. *Overholt BF, Lightdale CJ, Wang KK, et al. Photodynamic therapy with porfimer sodium for ablation of high-grade dysplasia in Barrett's esophagus: international, partially blinded, randomized phase III trial. Gastrointest Endosc 2005; 62: 488–98.*
11. *Barritt AS 4th, Shaheen NJ. Should patients with Barrett's oesophagus be kept under surveillance? The case against. Best Pract Res Clin Gastroenterol. 2008; 22: 741–50.*
12. *Fléjou JF. "Barrett's oesophagus: from metaplasia to dysplasia and cancer." Gut 2005; 54 Suppl 1: i6–12.*
13. *Spechler SJ. Columnar-lined esophagus. Definitions. Chest Surg Clin N Am 2002; 12: 1–13.*
14. *Gopal DV, Lieberman DA, Magaret N, et al. Risk factors for dysplasia in patients with Barrett's esophagus (BE): results from a multicenter consortium. Dig Dis Sci 2003; 48: 1537-41.*
15. *Pohler E, Craig AL, Cotton J, et al. The Barrett's antigen anterior gradient-2 silences the p53 transcriptional response to DNA damage. Mol Cell Proteomics 2004; 3: 534–47.*
16. *Murray E, McKenna EO, Burch LR, et al. Microarray-formatted clinical biomarker assay development using peptide aptamers to anterior gradient-2. Biochemistry 2007; 46: 13742–51.*
17. *Eloubeidi MA, Provenzale D. Clinical and demographic predictors of Barrett's esophagus among patients with gastroesophageal reflux disease: a multivariable analysis in veterans. J Clin Gastroenterol 2001; 33: 306–9.*
18. *Conio M, Filiberti R, Blanchi S, et al. Risk factors for Barrett's esophagus: a case-control study. International Journal of Cancer 2002; 97: 225–9.*

19. *Eloubeidi MA, Provenzale D. Does this patient have Barrett's esophagus? The utility of predicting Barrett's esophagus at the index endoscopy. Am J Gastroenterol 1999; 94: 937–43.*
20. *Kim SL, Waring JP, Spechler SJ, et al. Diagnostic inconsistencies in Barrett's esophagus. Department of Veterans Affairs Gastroesophageal Reflux Study Group. Gastroenterology 1994; 107: 945–9.*
21. *Curvers WL, Peters FP, Elzer B, et al. Quality of Barrett's surveillance in The Netherlands: a standardized review of endoscopy and pathology reports. Eur J Gastroenterol Hepatol. 2008; 20: 601–7.*
22. *Abrams JA, Kapel RC, Lindberg GM, et al. Adherence to biopsy guidelines for Barrett's esophagus surveillance in the community setting in the United States. Clin Gastroenterol Hepatol 2009; 7: 736–42.*
23. *Streitz JM Jr, Andrews CW Jr, Ellis FH Jr. Endoscopic surveillance of Barrett's esophagus. Does it help? J Thorac Cardiovasc Surg 1993; 105: 383–7.*
24. *Fountoulakis A, Zafirellis KD, Dolan K, Dexter SP, Martin IG, Sue-Ling HM. Effect of surveillance of Barrett's oesophagus on the clinical outcome of oesophageal cancer. Br J Surg 2004; 91: 997–1003.*
25. *Corley DA, Levin TR, Habel LA, Weiss NS, Buffler PA. Surveillance and survival in Barrett's adenocarcinomas: a population-based study. Gastroenterology 2002; 122: 633–40.*
26. *Cooper GS, Kou TD, Chak A. Receipt of previous diagnoses and endoscopy and outcome from esophageal adenocarcinoma: a population-based study with temporal trends. Am J Gastroenterol 2009; 104: 1356–62.*
27. *Kearney DJ, Crump C, Maynard C, Boyko EJ. A case-control study of endoscopy and mortality from adenocarcinoma of the esophagus or gastric cardia in persons with GERD. Gastrointest Endosc 2003; 57: 823–9.*
28. *Sampliner RE; The Practice Parameters Committee of the American College of Gastroenterology. Updated guidelines for the diagnosis, surveillance, and therapy of Barrett's esophagus. Am J Gastroenterol 2002; 97: 1888-95.*
29. *Inadomi JM, Sampliner R, Lagergren J, Lieberman D, Fendrick AM, Vakil N. Screening and surveillance for Barrett's esophagus in high-risk groups: A Cost–Utility Analysis. Ann Intern Med. 2003; 138: 176-86.*
30. *Mandal A, Playford RJ, Wicks AC. Current practice in surveillance strategy for patients with Barrett's oesophagus in the UK; observational study. Aliment Pharmacol Ther 2003; 17: 1319–24.*
31. *Society for Surgery of the Alimentary Tract. SSAT patient care guidelines: management of Barrett's esophagus. J Gastrointest Surg 2007; 11: 1213-15.*
32. *Montgomery E, Bronner MP, Goldblum JR, et al. Reproducibility of the diagnosis of dysplasia in Barrett's esophagus: a reaffirmation. Hum Pathol 2001; 32: 368- 78.*
33. *Sharma P, Falk GW, Weston AP, Reker D, Johnston M, Sampliner RE. Dysplasia and cancer in a large multicenter cohort of patients with Barrett's esophagus. Clin Gastroenterol Hepatol 2006; 4: 566-72.*
34. *Wani S, Mathur S, Sharma P. How to manage a Barrett's esophagus patient with low-grade dysplasia. Clin Gastroenterol Hepatol 2009; 7: 27-32.*
35. *Rastogi A, Puli S, El-Serag HB, Bansal A, Wani S, Sharma P. Incidence of esophageal adenocarcinoma in patients with Barrett's esophagus and high-grade dysplasia: a meta-analysis. Gastrointest Endos 2008; 67: 394-8.*
36. *Jobe BA, Hunter JG, Chang EY, et al. Office-based unsedated small-caliber endoscopy is equivalent to conventional sedated endoscopy in screening and surveillance for Barrett's esophagus: a randomized and blinded comparison. Am J Gastroenterol 2006; 101: 2693–2703.*
37. *Lin OS, Schembre DB, Mergener K, et al. Blinded comparison of esophageal capsule endoscopy versus conventional endoscopy for a diagnosis of Barrett's esophagus in patients with chronic gastroesophageal reflux. Gastrointest Endosc 2007; 65: 577–83.*
38. *Galmiche JP, Sacher-Huvelin S, Coron E, et al. Screening for esophagitis and Barrett's esophagus with wireless esophageal capsule endoscopy: a multicenter prospective trial in patients with reflux symptoms. Am J Gastroenterol. 2008; 103: 538–45.*
39. *Wolfsen HC, Crook JE, Krishna M, et al. Prospective, controlled tandem endoscopy study of narrow band imaging for dysplasia detection in Barrett's esophagus. Gastroenterology 2008; 135: 24-31.*
40. *Kiesslich R, Gossner L, Goetz M, et al. In vivo histology of Barrett's esophagus and associated neoplasia by confocal laser endomicroscopy. Clin Gastroenterol Hepatol. 2006; 4: 979-87.*
41. *Soni A, Sampliner RE, Sonnenberg A. Screening for high-grade dysplasia in gastroesophageal reflux disease: is it cost-effective? Am J Gastroenterol 2000; 95: 2086–93.*
42. *Inadomi JM, Sampliner R, Lagergren J, Lieberman D, Fendrick AM, Vakil N. Screening and surveillance for Barrett esophagus in high-risk groups: a cost-utility analysis. Ann Intern Med 2003; 138: 176–86.*
43. *Isenberg G, Sivak MV Jr, Chak A, et al. Accuracy of endoscopic optical coherence tomography in the detection of dysplasia in Barrett's esophagus: a prospective, double-blinded study. Gastrointest Endosc 2005; 62: 825–31.*
44. *Allison H, Banchs MA, Bonis PA, Guelrud M. Long-term remission of nondysplastic Barrett's esophagus after multipolar electrocoagulation ablation: report of 139 patients with 10 years of follow-up. Gastrointest Endosc 2010; 73 : 651-58.*
45. *Sharma P, Wani S, Weston AP, et al. A randomised controlled trial of ablation of Barrett's oesophagus with multipolar electrocoagulation versus argon plasma coagulation in combination with acid suppression: Long term results. Gut 2006; 55: 1233-9.*

46. *Sampliner RE, Faigel D, Fennerty MB, et al. Effective and safe endoscopic reversal of nondysplastic Barrett's esophagus with thermal electrocoagulation combined with high-dose acid inhibition: a multicenter study. Gastrointest Endosc 2001; 53: 554–58.*
47. *Van Laethem JL, Jagodzinski R, Peny MO, Cremer M, Devière J. Argon plasma coagulation in the treatment of Barrett's high-grade dysplasia and in situ adenocarcinoma. Endoscopy 2001; 33: 257-61.*
48. *Attwood SE, Lewis CJ, Caplin S, Hemming K, Armstrong G. Argon plasma coagulation as therapy for high-grade dysplasia in Barrett's esophagus. Clin Gastroenterol Hepatol 2003; 1: 258-63.*
49. *Kelty CJ, Ackroyd R, Brown NJ, Stephenson TJ, Stoddard CJ, Reed MW. Endoscopic Ablation of Barrett's Oesophagus: a randomized-controlled trial of photodynamic therapy vs. argon plasma coagulation. Aliment Pharmacol Ther 2004; 20: 1289-96.*
50. *Madisch A, Miehlke S, Bayerdorffer E, et al. Long-term follow-up after complete ablation of Barrett's esophagus with argon plasma coagulation. World J Gastroenterol 2005; 11: 1182-6.*
51. *Gondrie JJ, Pouw RE, Sondermeijer CM, et al. Effective treatment of early Barrett's neoplasia with stepwise circumferential and focal ablation using the HALO system. Endoscopy 2008; 40: 370-9.*
52. *Gondrie JJ, Pouw RE, Sondermeijer CM, et al. Stepwise circumferential and focal ablation of Barrett's esophagus with high-grade dysplasia: results of the first prospective series in 11 patients. Endoscopy 2008; 40: 359-69.*
53. *Sharma VK, Wang KK, Overholt BF, et al. Balloon-based, circumferential, endoscopic radiofrequency ablation of Barrett's esophagus: 1-year follow-up of 100 patients. Gastrointest Endosc 2007; 65: 185-95.*
54. *Ganz RA, Overholt BF, Sharma VK, et al. Circumferential ablation of Barrett's esophagus that contains high-grade dysplasia: a U.S. multicenter registry. Gastrointest Endosc 2008; 68: 35-40.*
55. *Fleischer DE, Overholt BF, Sharma VK, et al. Endoscopic ablation of Barrett's esophagus: a multicenter study with 2.5-year follow-up. Gastrointest Endosc 2008; 68: 867–76.*
56. *Johnston MH, Horwhat JD, Dubois A, Schoenfeld PS. Endoscopic cryotherapy in the swine esophagus: a follow up study [Abstract]. Gastrointest Endosc. 1999; 49: AB126.*
57. *Pasricha PJ, Hill S, Wadwa KS, et al. Endoscopic cryotherapy: experimental results and first clinical use. Gastrointest Endosc 1999; 49: 627-31.*
58. *Raju GS, Ahmed I, Xiao SY, brining D, Bhutani MS, Pasricha PJ. Graded esophageal mucosal ablation with cryotherapy, and the protective effects of submucosal saline. Endoscopy 2005; 37: 523-6.*
59. *Halsey KD, Chang JW, Waldt A, Greenwald BD. Recurrent disease following endoscopic ablation of Barrett's high-grade dysplasia with spray cryotherapy. Endoscopy 2011; 43: 844-8.*
60. *Greenwald BD, Dumot JA Cryotherapy for Barrett's esophagus and esophageal cancer. Curr Opin Gastroenterol 2011; 27: 363–7.*
61. *Cheon YK. Metal stenting to resolve post-photodynamic therapy stricture in early esophageal cancer. World J Gastroenterol 2011; 17: 1379-82.*
62. *Overholt BF, Wang KK, Burdick JS, et al. Five-year efficacy and safety of photodynamic therapy with photofrin in Barrett's high-grade dysplasia. Gastrointest Endosc 2007; 66: 460-8.*
63. *Wolfsen HC, Hemminger LL, Wallace MB, Devault KR. Clinical experience of patients undergoing photodynamic therapy for Barrett's dysplasia or cancer. Aliment Pharmacol Ther 2004; 20: 1125-31.*
64. *Prasad GA, Wang KK, Halling KC, et al. Utility of biomarkers in prediction of response to ablative therapy in Barrett's esophagus. Gastroenterology. 2008; 135: 370-9.*
65. *Overholt BF, Panjehpour M, Halberg DL. Photodynamic therapy for Barrett's esophagus with dysplasia and early carcinoma: long-term results. Gastrointest Endosc 2003; 58: 183-8.*
66. *Pech O, Behrens A, May A, et al. Long-term results and risk factor analysis for recurrence after curative endoscopic therapy in 349 patients with high-grade intraepithelial neoplasia and mucosal adenocarcinoma in Barrett's oesophagus. Gut 2008; 57: 1200-6.*
67. *Peters FP, Brakenhoff KP, Curvers WL, et al. Histologic evaluation of resection specimens obtained at 293 endoscopic resections in Barrett's esophagus. Gastrointest Endosc 2008; 67: 604–9.*
68. *Prasad GA, Buttar NS, Wongkeesong LM, et al. Significance of neoplastic involvement of margins obtained by endoscopic mucosal resection in Barrett's esophagus. Am J Gastroenterol 2007; 102: 2380–6.*
69. *Ell C, May A, Pech O, et al. Curative endoscopic resection of early esophageal adenocarcinomas (Barrett's cancer). Gastrointest Endosc 2007; 65: 3-10.*
70. *Peters FP, Kara MA, Rosmolen WD, et al. Stepwise radical endoscopic resection is effective for complete removal of Barrett's esophagus with early neoplasia: a prospective study. Am J Gastroenterol 2006; 101: 1449-57.*



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# Instructions for the authors

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**Abstract**

In this paper the instructions for preparing camera ready paper for the Journal are given. The recommended, but not limited text processor is Microsoft Word. Insert an abstract of 50-100 words, giving a brief account of the most relevant aspects of the paper. It is recommended to use up to 5 key words.

**Key words:** Camera ready paper, Journal.

## Introduction

In order to effect high quality of Papers, the authors are requested to follow instructions given in this sample paper. Regular length of the papers is 5 to 12 pages. Articles must be proofread by an expert native speaker of English language. Can't be accepted articles with grammatical and spelling errors.

## Instructions for the authors

Times New Roman 12 points font should be used for normal text. Manuscript have to be prepared in a two column separated by 5 mm. The margins for A4 (210×297 mm2) paper are given in Table 1.

*Table 1. Page layout description*

| Paper size | A4 |
|---|---|
| Top margin | 20 mm |
| Bottom margin | 20 mm |
| Left margin | 20 mm |
| Right margin | 18 mm |
| Column Spacing | 5 mm |

Regular paper may be divided in a number of sections. Section titles (including references and acknowledge-ment) should be typed using 12 pt fonts with **bold** option. For numbering use Times New Roman number. Sections can be split in subsection, which should be typed 12 pt *Italic* option.

Figures should be one column wide. If it is impossible to place figure in one column, two column wide figures is allowed. Each figure must have a caption under the figure. Figures must be a resolution of 300 DPI, saved in TIFF format, width 10 cm min. For the figure captions 12 pt *Italic* font should be used. (1)

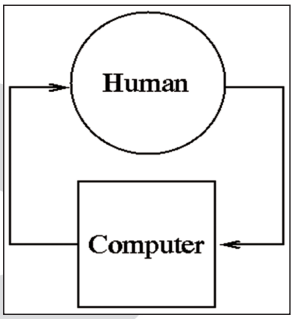


*Figure 1. Text here*

## Conclusion

Be brief and give most important conclusion from your paper. Do not use equations and figures here.

## Acknowledgements (If any)

These and the Reference headings are in bold but have no numbers.

## References

1. *Sakane T, Takeno M, Suzuki N, Inaba G. Behcet's disease. N Engl J Med 1999; 341: 1284–1291.*
2. *Stewart SM, Lam TH, Beston CL, et al. A Prospective Analysis of Stress and Academic Performance in the first two years of Medical School. Med Educ 1999; 33(4): 243- 50.*

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