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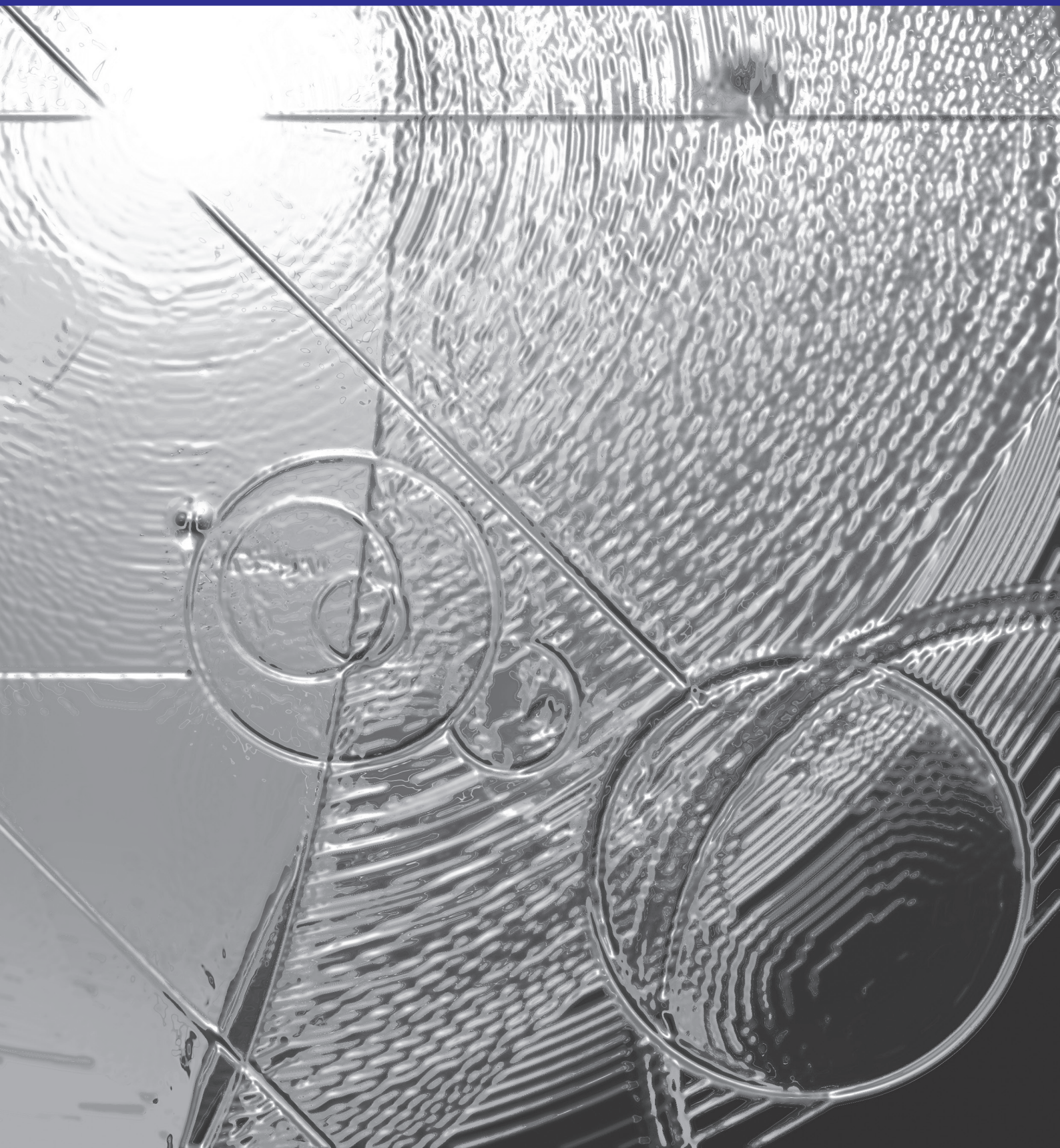

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Anxiety and Depression in Pakistani Medical Students: A Multi-Center Study

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Abstract

Objective: To determine the prevalence of anxiety and depression symptoms and associated risk factorsin medical students in Pakistan.

Methodology: This was across-sectional study conducted at six medical schools in Pakistan.Medical students of all years were included. Data was collected by a team of medical students at each institution through convenience sampling. A self-administered structured questionnaire was prepared for data collection and distributed among medical students. Students were asked about basic demographic details and information was acquired about known and anticipated associated risk factors. Depression and anxiety symptoms were assessed using The Aga Khan University Anxiety and Depression Scale (AKUADS), a validated scale in Urdu developed for the Pakistani population.

Results: A total of 437 students participated in the study. Anxiety and Depression symptoms were present in 45.5% of students defined as a score of ≥20 on AKUADS. Mean AKUADS score of whole sample was 21.3± 15. Factors significantly associated with anxiety and depression symptoms in multivariate analysis were medical school year, failure in medical school annual examination, number of hours of study, family history of depression and past history of depression.

Conclusions: There is a high prevalence of depression and anxiety symptoms in medical students, making them a high-risk, vulnerable group. This reflects the need for widespread implementation of remedial measures by medical school administrations, including education, assessment and provision of mental health services.

Key words: Depression, Anxiety, Medical Students, Cross-sectional Study

Introduction

"In the end, only the wounded physician heals and even he, in the last analysis, cannot heal beyond the extent to which he has healed himself." Carl Jung

Medical schools are known to be highly stressful environments and the mental health of medical students has been a subject of interest to many researchers, going as far back as the 1950s[1]. A number of studies have reported a high prevalence of depression and anxiety among medical students, both internationally and from Pakistan.

The Global Perspective

A systematic review of 40 studies examining depression and anxiety in medical students from colleges in USA and Canada[2] reported prevalence rates mostly in the 20% range, though a study by Dyrbye et al. (sample size of 3080) reported the prevalence of depressive symptoms to be 49%[3]. In a multicenter study of more than 2000 medical students and residents in USA, 12% had probable major depression, and an additional 9.2% had symptom levels consistent with mild to moderate depression[4]. Studies from some other countries report similar prevalence figures of anxiety and depression, for example: Saudi Arabia (57%)[5], Iran (53%)[6] and Egypt[7] (49.5%). A study from Sweden[8] found the prevalence to be 12.9%. (The relatively lower prevalence reported from Sweden may be a consequence of the over-all social, political and economic stability of the country, but this is a conjecture on our part.)

Pakistan

A literature review identified four other studies on depression and anxiety in medical students conducted in Pakistan[9-12]. Two of them are from Kara-

chi, one from Multan and one from Lahore. All of them are single center studies. The one in Lahore surveyed only female medical students. Three of these studies employed The Aga Khan University Anxiety and Depression Scale (AKUADS), while one study utilized the Hospital Anxiety and Depression Scale (HADS). Significant findings from these studies are summarized in Table 1. The study by Jadoon et al.[10] in Multan reported a prevalence of 43.89% while the other three studies have reported a higher prevalence (60-70%)[9,11-12].

AKUADS

The Aga Khan University Anxiety and Depression Scale (AKUADS) is a validated scale in Urdu language developed for the Pakistani population. This scale was developed from a list of actual complaints that patients presented with, and is therefore uniquely representative of local symptom complexes[13]. It is a 25-item scale, assessing 12 psychiatric and 13 somatic symptoms, and provides an aggregate score for depression and anxiety. The AKUADS offers superiority over Urdu translations of depression scales developed in the West because it is locally developed, culturally appropriate, and valid in both local and international comparisons[14].

AKUADS score of 20 as a cut-off is 66% sensitive and 79% specific, and has 83% positive predictive value (PPV) and 60% negative predictive value (NPV)[14]. Using a score of 19 increases the sensitivity to 74%, but lowers the positive predictive value (PPV) to 61%[13]. Therefore a cut off score of 20 is deemed to be the best trade-off. The sensitivity, specificity, PPV and NPV refer to the presence of anxiety disorders (including Generalized Anxiety Disorder and Panic Disorder) and Major Depressive Disorder as diagnosed on structured clinical interview by a psychiatrist according to DSM-III-R criteria. The scale is self-administered and does not require the assessment of a psychiatrist for validity. AKUADS has a high internal consistency, given that all its items contribute to the total score[15]. It is designed to be used in epidemiological and community studies to screen individuals with anxiety and depression symptoms.

Aims and objectives of our study

The objective of our study was to estimate the prevalence of anxiety and depressive symptoms in medical students of Pakistan at multiple centers using the appropriate AKUADS cut-off of 20. Our study contributes to the prior literature on anxiety and depression in Pakistan medical students by revealing whether the high prevalence and associated risk factors reported can be replicated in a multi-center setting using the appropriate cut-off.

Anxiety disorders and major depressive disorder are clinically diagnosed with DSM criteria (latest version of which is DSM-5), and these psychiatric diagnoses cannot be made using a screening tool. Given that AKUADS is a self-administered screening questionnaire, and given that it provides us with an aggregate score for anxiety and depression, it cannot be used for prevalence calculations of anxiety and depressive disorders. We have utilized the AKUADS to determine the prevalence of anxiety and depressive symptoms in medical students. For the purpose of comparison with prevalence figures

Table 1. Literature Review: Anxiety and Depression in Pakistani Medical Students

Study	Scale Used	Sample Size	Prevalence of Anxiety and Depression	Significant Associations
Inam et al. (2003)[9]	AKUADS	189	60%	Year of medical college
Khan et al. (2006)[11]	AKUADS	142	70%	Substance abuse Family history Death of relatives Year of medical college
Rab et al. (2008)[12]	HADS	87	Anxiety: 43.7% Depression: 19.5%	Recent major life events Year of medical college Dormitory residents Number of friends
Jadoon et al. (2010)[10]	AKUADS	482	43.89%	Female gender Year of medical college

of anxiety and depressive disorders, we use the AKUADS as an indirect measure of the aggregate prevalence of anxiety and depressive disorders (given its high PPV). AKUADS results are not the actual prevalence of these disorders and they should not be treated so. Previous studies conducted in Pakistan have utilized AKUADS for prevalence calculations without explicitly acknowledging this distinction between the prevalence of symptoms and disorders.

Methodology

This cross-sectional survey was conducted at six medical schools in Pakistan from August 2012 to September 2013. These six institutions are King Edward Medical University (Lahore), CMH Lahore Medical and Dental College (Lahore), Sharif Medical College (Lahore), Allama Iqbal Medical College (Lahore), Services Institute of Medical Sciences (Lahore) and Baqai Medical College (Karachi). These medical schools were selected because of availability of manpower in those schools that could collect data, while representinga diversity of socio-economic and ethnic groups at the same time.Four of them are public medical schools and two are private. Ethical approval from the Institutional Review Board (IRB) of all medical schools was obtained. All medical students of five MBBS classes (from 1st year MBBS to 5th year MBBS) were eligible to participate in the study. Data was collected by a team of medical students at each medical school through convenience sampling. A self-administered structured questionnaire was prepared for data collection and distributed among the medical students. Students were approached in person.Filling out the questionnaire implied consent for participation in the survey. Students were asked about basic demographic details like name, age, gender, residence, and year of medical school. The questionnaire was anonymous to maintain the privacy of participants.Information was also acquired about personal and family history of depression, current use of antidepressant drugs, average number of hours per day spent studying, and failure in the most recent annual examination.

Statistical Package for Social Science (SPSS) version 20 was used for data entry and analysis. Frequencies and percentages were calculated for qualitative variables like gender, failure in the most recent annual examination, and a personal and family history of depression. Means and standard deviations were calculated for quantitative variables like age, number of hours per day spent studying etc. A score of ≥20 on AKUADS was used as a cut-off for the presence of anxiety and depressive symptoms. Bivariate analysis was done to identify factors associated with anxiety and depression. P value <0.05 was considered significant. Ethical concerns were considered by the research team regarding whether provisions for further psychiatric management could be provided to those students who had scored above the cut-off for depression and anxiety. However, given that the questionnaire was anonymous, there was no way for us to link individual responses to individual students. Therefore all participants were given general psychoeducation regarding anxiety and depression, and were encouraged to seek psychiatric consultation if they felt the symptoms were causing them significant distress and impairment.

Results

Baseline characteristics

A total of 437 students from six medical schools participated in the study. Females comprised 59% (258/437) of participants. Mean age of students was 21 ± 1.9 years. A majority of students (54.7%) were residing in college hostels. 15.6%, 19.7%, 22.9%, 16% and 25.9% of the students were from 1st, 2nd, 3rd, 4th, and 5th year of medical school respectively. (Table 2)

Table 2. Demographics

Sample Size		437
Gender	**Males**	179 (41%)
	Females	258 (59%)
Mean Age		21 ± 1.9 years
Residence	**Hostel**	239 (54.7%)
	Home	198 (45.3%)
Year of Study	**1**	68 (15.6%)
	2	86 (19.7%)
	3	100 (22.9%)
	4	70 (16%)
	5	113 (25.9%)

A vast majority of students (84.9%) had not had any failure in medical school annual examinations.

Anxiety and depressive symptoms were present in 45.5% of students defined as a score of ≥20 on AKUADS. Mean AKUADS score of whole sample was 21.3 ± 15. Personal and family history of depression was reported by 10.9% and 16.7% of students respectively. 3.5% students were already taking anti-depressants.

Bivariate analysis

Students with and without anxiety and depression symptoms did not differ significantly regarding mean age (20.9 ± 2.1 years vs 21.1 ± 1.8 years, p value 0.32). Comparison of number of hours of study per day showed that students withanxiety and depression symptoms spent more hours studying than non-depressed students (3.3 ± 3.3 hours vs 2.5 ± 1.9 hours, p <0.01). Students from junior classes were more likely to have anxiety and depression symptoms than students from senior classes (P value for trend 0.02). A higher proportion of with anxiety and depression symptoms students had a family history of depression compared to non-depressed students (21.8% vs 12.4% respectively, P value <0.01). Students with a personal history of depression were 5.2 times more likely to have a score of ≥20 on AKUADS than students without personal history of depression (P value<0.01). Students who had a history of failure in any annual examination during medical school were significantly more anxious and depressed than students who did not have such failures (62.5% vs 41.5%, P value <0.01). Students with and without anxiety and depression symptoms were not different regarding gender, residence, and current use of anti-depressants. (Table 3)

Discussion

We found the prevalence of anxiety and depressive symptoms in our sample of medical students to be 45.5%. This is consistent with thefindings by Jadoonet al.[10] in Multan where a prevalence of 43.89% was reported in a sample size of more than 400 students. Other studies have reported a higher prevalence (60-70%), which may be a consequence of their smaller sample sizes. Another factor to consider when comparing these different studies is that in the three studies in which the AKUADS was employed, a cut off score of 19 or greater was used, in comparison to our study which used a score of 20 or greater as the cut off. As discussed before, using a score of 19 increases the sensitivity, but significantly lowers the positive predictive value (PPV)[13]. The higher prevalen-

Table 3. Association of different variables with depression in medical students

Variable		Depressed	Not-depressed	P value
Gender (n)	Male	72	107	0.06
	Female	127	131	
Age in years (mean)		20.9	21.1	0.32
Hours/day studied (mean)		3.3	2.5	**<0.01**
School Year (n)	1st	44	24	**0.02**
	2nd	35	51	
	3rd	43	57	
	4th	30	40	
	5th	47	66	
Residence (n)	Hostel	102	137	0.18
	Home	97	101	
Exam Failure (n)	Yes	40	24	**<0.01**
	No	150	211	
Family history of depression (n)	Yes	43	29	**<0.01**
	No	154	205	
Personal history of depression (n)	Yes	37	10	**<0.01**
	No	159	226	
Taking anti-depressants (n)	Yes	10	5	0.09
	No	186	231	

ce reported by other studies may therefore be in part because of the higher sensitivity (but lower PPV). Other possible causes may be differences in teaching methods, college environments, sociopolitical considerations and differing demographics.

The reasons for the high prevalence of psychological distress in medical students can be attributed to excessive stress of studies, a higher academic burden in terms of syllabus, courses and required competence, lack of leisure time, and high competition[16, 17]. Added factors for Pakistan may be frustrations related to the educational and work environment, attitudes of teaching faculty and the prospect of limited opportunities for post-graduation training with limited pay. In a recent study of medical students in Pakistan, 78% of students reported being subjected to gender discrimination, which led to anger, frustration and helplessness[18]. This may also contribute to the prevalence of anxiety and depression in medical students.

Factors found to have a significant association with depression in medical students in our study were year of medical college, hours of study, family history of depression, personal history of depression and failure in medical school annual examinations. The association of depression and anxiety with the year of medical college is one finding which is consistent across all studies done in Pakistan. Higher rates are seen in medical students of first and second year. This may be explained by the fact that medical students in the first two years have to adjust to a new and highly stressful academic environment.The difficulty and complexity of the course material is also higher compared to clinical subjects and the attitudes of the basic science teachers is also said to be more stringent and harsh. The association with medical year has also been found in two studies from USA, which reported that peak depression scores were found at the end of the second year but the depression scores remained higher than baseline scores throughout the duration of the study[19, 20].

Association of a positive family history with depression has been reported by Khan et al.[10] as well. The associations with hours of study, personal history of depression and failure in medical school annual examinations are findings that have not been reported previously from Pakistan. These are being reported for the first time. A positive family of depression and a past history of depression are well-known risk factors for major depressive disorder[21]and therefore their association in our sample of medical students is not surprising. The association with hours of study is difficult to explain, given that we do not know the casual relationship. It could be that anxious and depressed students spend more time studying because their depressed cognition slows the process of learning, and they have to spend more time learning the same material compared to a student who is not anxious or depressed. Medical education in Pakistan is generally based on a traditional teaching and assessment methods with end of year written and oral examinations. Students require 50% marks in both written and oral exams separately for passing on to the next medical education year. Failure in the annual examinations requires one to retake them as supplementary examinations, failing which they are required to repeat the entire year of medical education. Failure in the annual examination is therefore the highest degree of academic stress a medical student can face. The association of stressful events with the development of anxiety and depressed is also well-recognized[21]. This finding should serve as a recommendation for a change in the system of evaluation of medical students, such that performance throughout the year should determine promotion to the next level rather than a single end of the year examination.

The study by Jadoonet al.[10] reveals a higher prevalence of depression in females.Our study found a higher prevalence of depression and anxiety in female medical students compared to male medical students, but this was not statistically significant (p-value 0.06). Studies from USA and Canada have reported mixed findings regarding gender as well, with 4 of 5 cross-sectional studies and 3 of 7 longitudinal studies included in the systematic review by Dyrbye et al.[2] reporting no difference in depression scores by gender.

Students with and without anxiety and depression symptoms were not different with regards to the current use of anti-depressants, probably because the number of students on anti-depressants was too small (3.6%). The fact that about 45.5% of students had anxiety and depressive symptoms but only 3.6% were on antidepressants at the time of study reveals that only a small percentage of

medical students with anxiety and depression had soughtpsychiatric assistance and had received treatment.Given that the presence of depression in medical students significantly impacts their life satisfaction[22], increases the risk of suicide[23] and serves as a predictor of problems with patient care later in professional life[24], it is highly recommended that medical schools should take definitive measures to address this problem. At the very least, all students should have access to a mental health counselor with whom they may discuss their problems and who can identify students who are depressed and anxious, and those who require psychiatric intervention in the form of psychotherapy and medication.

Limitations

While this was a multi-center study, it did not include medical schools from all provinces of the country. Future multi-center studies should attempt to aim for representation from all provinces.Our sample size is still quite small given the large number of medical students that are enrolled in the six medical schools we conducted this study at.A larger sample size would have further increased the power of the study, and may have uncovered more significant associations, such as gender. Convenience sampling is another limitation, as it limits the representativeness of the sample. Data about the number of medical students approached and response rate is not available. As the study design was cross-sectional, cause-effect associations cannot be determined. Given that the study did not have a control group, there is no way to compare the prevalence of depression in medical students with that of either the general population or that of students in other professional fields, e.g. Law or Business. In addition, we had no information on the baseline mental health status of medical students at the time of their admission.

Depression in Medical Students: What Are We Missing?

For the study conclusions to be meaningful, they need to be discussed in comparison to the depression prevalence and risk factors found in the general Pakistani population. In 2004 Mirza and Jenkins published a systematic review of 20 studies giving prevalence estimates and risk factors of anxiety and depression in Pakistan. Mean overall prevalence of anxiety and depressive disorders in Pakistani population was found to be 34% (range 29-66% for women and 10-33% for men)[25]. We found the prevalence of anxiety and depressive symptoms in medical students to be 45.5%. Using a positive predictive value of 83%, we can estimate the prevalence of anxiety and depressive disorders in medical students in our study to beabout 38%. This would indicate that the prevalence of anxiety and depression in medical students is comparable to that of general population.However, this can only be validated by a direct comparison study of medical students with the general population, and by a study that uses clinical interview for assessing prevalence of anxiety and depressive disorders. A cross-sectional study with the general population as a control is needed to examine the validity of this difference, anda case control study would be required to assess whether medical student status serves as an independent risk factor for depression.

Another important factor required for a meaningful analysis is the prevalence of anxiety and depression in students of other professions, such as engineering, law and humanities. Literature reviews indicate that no such study has been conducted in Pakistan. All available studies are on medical students, probably because of their ease of access to medical authors. Unless such studies are available, it cannot be concluded whether the high prevalence of depression and anxiety in medical students is unique to medical training or simply a characteristic of professional university education in general. Although the available literature is limited and constrained by methodological issues, comparative studies conducted in USA report higher prevalence rates of depression and anxiety in pharmacy students[26], graduate science students[27, 28] and law students[29] compared to medical students. A study from UK[30] reported the prevalence of depression among medical students to be similar to that reported for comparable groups. Another study from UK[31] found that non-medical students showed a higher prevalence of moderate and severe depressive symptoms than their medical student peers, although medical students had a

higher prevalence of mild depressive symptoms. A study from Australia[32] revealed that law students showed elevated levels of distress compared to medical students. The existence of this comparative literature reflects the need for further study of this kind in Pakistan as well. This discussion is not intended to downplay the distress and severity of the depression experienced by medical students noras a dismissal of the urgent need for remedial interventions. At the same time, however, it is important to point out that isolated data about medical students without comparison with other specialties tells us little about how unique this problem is to the medical profession in Pakistan.

To address this problem more effectively in the future, apart from comparative studies with control groups, longitudinal studies, which would assess the change in prevalence of depression and anxiety in a sample of medical students as they pass through each year, and interventional studies, which would evaluate the change in the prevalence of depression and anxiety among medical students after the implementation of remedial measures, such accessibility to counselors, are necessary.

When the high prevalence of depression was reported by Goebert et al. in their multi-school study from the USA, Reynolds and Clayton in their commentary on the article[33] declared it 'a clarion call to action on the part of leadership in the nation's schools of medicine and academic health centers'. We hope that our study will serve to do the same for the medical students of Pakistan.

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Authorship Rank

For author ranking of this paper, we use the informal but accepted norm of "first-last-author-emphasis", where the first author is the chief contributor to the research and manuscript writing while the last author provides crucial mentorship role and is the intellectual driving force behind the project.

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Hepatic differentiation of human umbilical cord derived mesenchymal stem cells (hMSCs) on alginate scaffold

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Abstract

Introduction: Tissue engineering using stem cell has been proposed as an alternative to liver transplantation to cure liver diseases. We hypothesized that MSCs obtained from Wharton jelly are capable of differentiating into functional hepatocyte-like cells in Alginate scaffold.

Material and methods: Isolated of MSCs from wharton jelly explants divided into 2D and 3D culture in alginate scaffold. Surface antigens were analyzed by flow cytometry (CD73, CD31). Hepatic differentiation UC-MSCs was performed by four step sequential method: 10 ng/ml FGF-4,1%ITS, 20 ng/mlHGF,100Nm dexamethasone 10−7 M glucagon, 10 ng/ml OSM,100nM TSA. Urea and Albumin concentration was quantified by ELISA.

Results: Isolated MSCs expressed mesenchymal markers such as CD73, but were negative against CD31. Polygonal morphology, cellular granularity and intracyplasmic inclusion bodies in 2D culture. Tissue construct of 3D culture revealed multicellular tissue with round shape cells resembled liver like tissue arranged in hepatocyte like plates. Albumin and Urea production was significantly higher in the experimental group of 3Dculture. However treatment of cells with TSA in final step, led to decrease albumin production. Exposure of cells to TSA at day14 led to increase of CK18 expression and decreae of αFP expression in both groups. But Exposure of cells to TSA at day14 led to decreased albumin gene expression in 3D culture.

Discussion: Results of the present study indicated that sequential exposure with TSA at the final step suppress albumin gene expression and albumin production activity in differentiated mesenchymal stem cell cultured in alginate scaffold.

Key words: Umbilical cord, mesenchymal stem cells, alginate scaffold, liver, hepatic differentiation

Introduction

The liver is one of the most important organ in the body and it is responsible for more than 500 functions. The function of liver grouped as four general categories: drug detoxification, energy metabolism, protein synthesis and bile production. Thus Without a healthy liver, a person cannot survive. Liver damage can impact almost all body systems and may progress to liver failure and emergency liver transplantation. But liver transplantation is limited by the organ donor availability (Carbone & Lerut, 2012). Tissue engineering using hepatocyte has been proposed as an alternative to liver transplantation (Muraca & Gerunda, 2002). Due to limitations of mature hepatocyte availibility, alternative cell sources are being pursued. Human mesenchymal stem cells (hMSCs) have been used in cell therapy and tissue engineering as a source of regenerating cells. Among stem cells, hMSCs derived from Wharton jelly are an obvious source of multipotential cells and can be easily isolated from umbilical cord.

Different natural and synthetic scaffolds have been used for liver tissue engineering. Among different scaffolds tested for tissue regeneration, alginate has great advantages such as biocompatibility, ability to increase the viscosity, ability to form gels, form films of sodium/calcium alginate and calcium alginates fibers and is used for surgical dressings, and delivery of proteins, and cell delivery (Dvir-Ginzberg&Gamlieli-Bonshtein,2003; McHugh,2003). But in limited previous studies alginate was used for encapsulation of in liver tissue

engineering (Maguire & Davidovich, 2007; Lin & Lin, 2010; Zhang & Wan, 2011).

One of the most important factors in liver tissue engineering is methods of hepatic differentiation. Different methods categorized into two group: cocktail and sequential manner. Different sequential methods examined for hepatic differentiation. Snykers (2011) and Yoon (2011) examined four step differentiation methods (Yoon & Jung, 2010; Snykers & De Kock, 2011), Waclawczyk and Campard examined three step sequential method (Waclawczyk & Buchheiser et al., 2010; Campard & Lysy et al.,2008), Zhao and Tamagawa (Tamagawa & Oi et al., 2007, Zhao & Ren et al.,2010) examined cocktail exposure of growth factors and cytokine on hepatic differentiation of stem cell. Many of these studies performed in 2D Culture condition.

The aim of this study was to investigate the hepatic differentiation potential of human MSCs. Therefore we hypothesized that MSCs obtained from Wharton jelly are capable of differentiating into functional hepatocyte-like cells in Alginate scaffold. We aimed to compare hepatic differentiation of human MSCs in 2D Culture condition and more innovative, in a sequential manner in 3D culture closely reflecting in vivo condition (Figure 1).

Material and methods

Materials

Phosphate-buffered saline (PBS), fetal bovine serum (FBS), Dulbecco's Modified Eagle Medium (DMEM), antibiotic (penicillin–streptomycin), L-glutamine, Collagenase Type I, ascorbic acid 2-phosphate glycerophosphate, Insulin, Hyaluronidase, trypsin-EDTA, alginate and trypsin, HEPES, NaCl and CaCl2 were purchased from Sigma-Aldrich (MO, USA).

Most biochemicals for hepatic differentiation including dexamethasone, Transferrin, Selenium (ITS), glucagon, Trichostatin A (TSA), Dimethyl sulfoxide (DMSO) ,Growth factors/cytokine, Fibroblast Growth Factor 4 (FGF4), Hepatocyte growth factor (HGF), Oncostatin M (OSM) were purchased from Sigma-Aldrich (MO, USA).Urea and albumin assay kits were purchased from BioAssay Systems (California, USA). Chemicals for RT-PCR were purchased from Qiagen (CA, USA). Antibodies against surface antigens, anti-CD73,CD31 were purchased from Biosciences (San Diego, CA). All chemicals were used directly without further purification.

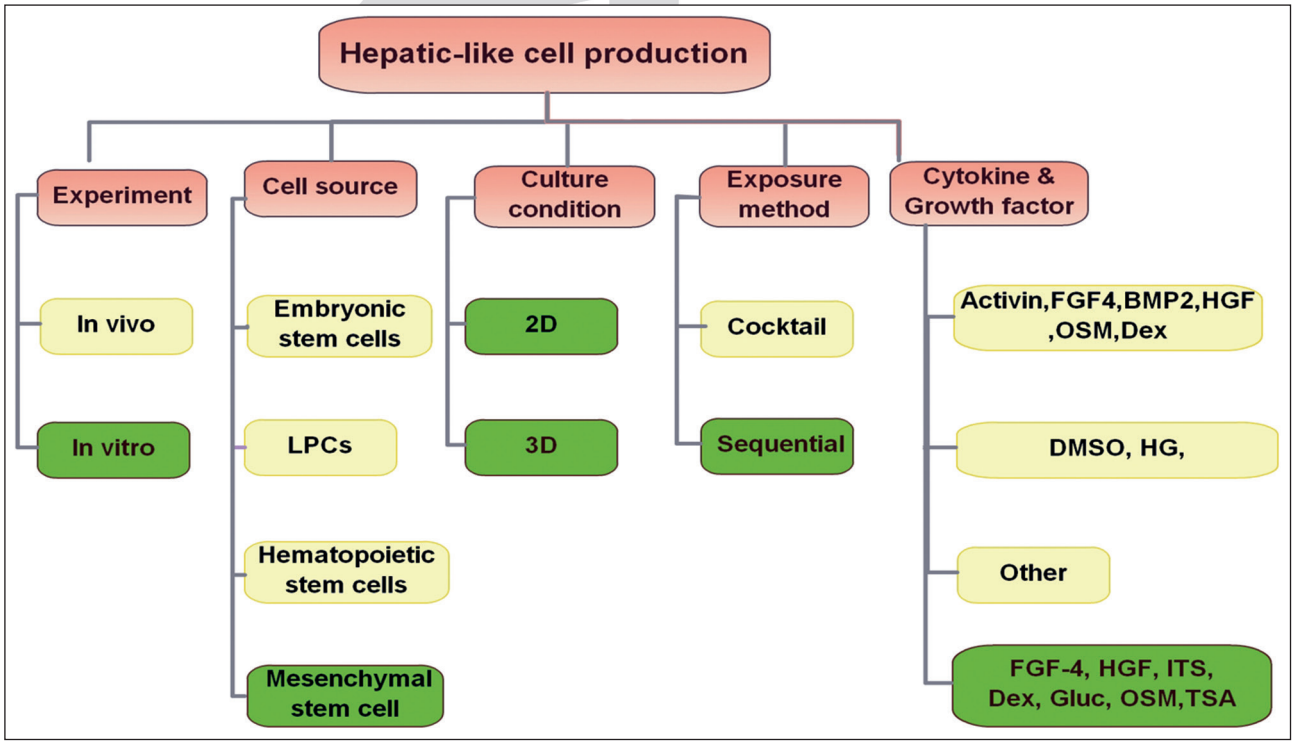

Figure 1. Study design and selection of hepatic differentiation method from available approaches. Our selection indicated by green color

Ethical approval

The study protocols was approved by the Ethics Committee of Ahvaz Jundishapour University of Medical Sciences (AJUMS) After getting ethical approval, UC cells were harvested from the umbilical cords of healthy full-term infants born by cesarean section (C/S). Informed consent was completed by the mothers prior to collection of UC. The collection of umbilical cord was performed in the Departments of Obstetrics and Gynecology at the Imam Khomeini in Jundishapour University of Medical Sciences Ahvaz, Iran (AJUMS). Since at birth the umbilical cord is approximately 50cm in length, 20 cm length pieces cut from each umbilical cord and were transferred to sterile containers containing sterile transport medium (DMEM (low glucose) containing 100 U/mL penicillin, 100 mg/mL streptomycin, and 0.25 mg/mL amphotericin B. and stored at 4°C for 2 - 6 hrs before further processing and were transported to laboratory of Cellular and Molecular Research Center (CMRC), Department of Anatomical Sciences, Faculty of Medicine, Ahvaz Jundishapour University of Medical Sciences (AJUMS).

Wharton jelly dissection

The umbilical cord was cut into smaller 2 - 4-cm length pieces. The pieces was washed carrying solution, and repeated 2 or 3 times. Trapped blood within the umbilical blood vessels removed. By horizontal sections at the external surface, outer sheath of umbilical cord cut and its inner surface containing the WJ extracted,vein and arteries of WJ were removed. Extracted WJ was cut into 3 - 5-mm pieces.

Isolation of MSCs from wharton jelly explants

6 - 9 pieces of Wharton jelly were transferred onto tissue culture 25-cm2 T-flasks containing complete culture medium (CCM) composed of DMEM (low glucose) with 2 mM L-glutamine, supplemented with 20% fetal bovine serum (FBS), 100 U penicillin/streptomycin. The pieces were left at the bottom of the flasks and placed in a 37°C in a humidified atmosphere containing 5% CO2. After 7-10 days, the culture medium was removed and thereafter changed twice a week.

Cell Expansion

Cells were allowed to adhere to culture flask and non-adherent cells were washed off and the culture medium was replaced. The culture takes approximately one week to reach confluence covering 70% - 80% of the flask. Then the medium was removed and were washed once with PBS and trypsinized with 0.25% trypsin-EDTA in an incubator for 5 minutes at 37° C. Culture examined for evidence of detachment of the adherent layer. Detached cells floating in the medium were centrifuged at 200 - 300 g for 10 min, the supernatant aspirated and discardded, cell pellets washed with 2 to 5 ml of fresh growth medium and transfered the resuspended cells to a 25 cm2 flask flask at a density 250 × 103 cells/cm2. During subculture, they were passaged every 5 days; medium was replaced every 3-4 days or twice a week. The cells Subcultured when they were reached 80 to 90% confluent (about 7-10 days)

Flow cytometry

After trypsinization and harvesting cells, 5 × 105 cells were resuspended in PBS and 5% FBS incubated with phycoerythrin (PE)- or fluorescein isothiocyanate (FITC)-conjugated antibodies against CD73, CD31 and IgG (ebio science, USA) for 30 min at dark room at 4°C. Labeled cells were assayed by Dako Glaxy flocytometer. Mouse isotype-matched Antibody (Ab) were used as controls (ebio science, USA)

Hepatic differentiation of UC-MSC in Experimental group

Monolayer culture

UC-MSCs were seeded into 25-cm2 T-flasks at a density of 1×104 cells/cm2. Hepatic differentiation of UC-MSC was induced with a sequential 4-step protocol (9).

Step I: DMEM (low glucose)+ 10 ng/ml FGF-4

Step II:DMEM (low glucose) + 1%ITS + 20 ng/mlHGF

Step III: Williams' E medium + 100Nm dexamethasone + 1% ITS + 10−7 Mglucagon + 10 ng/ml OSM

Step IV: Step III medium±100nM TSA or 1% DMSO] (Figure 1). The media was exchanged every 2-3 days

3D culture

MSCs Encapsulation in Hydrogel Scaffold
Preparing alginate solution (Alginate of 2.0% w/v)

Alginic acid sodium salt was added into the 0.15 M NaCl, and 0.025 M HEPES in deionized water by stirring and heating and sterilized via 0.45-nm filter [14]. MSCs with density of 1× 106 cells/ml were resuspended in 2 - 5 ml prepared solution, and then dropped into a 102-mM CaCl2 solution. Encapsulated MSC in alginate hydrogel were allowed to polymerize for a period of 10 minutes in the CaCl2 solution. Complete polymerization required up to 10 minutes. Then alginate beads containing MSCs washed 2 - 3 times 10 volumes of 0.15 M NaCl. These tissue constructs finally placed in 25-cm2 T-flasks with complete culture medium. Hepatic differentiation of UC-MSC was induced with a 4-step protocol as described above.

Urea assay

Supernatants of culture collected from each differentiation steps and Urea concentration was quantified by urea assay kit according to manufacturer's instruction.

Albumin assay

Supernatants of culture collected from each differentiation steps and Albumin secretion in the culture medium was quantified by ELISA according to the manufacturer's instructions

PCR

Gene expression level of AFP, ALB, CK18 were determined by RT–PCR.

Primers were first designed using Primer Express 1.0a Software (Applied Biosystems) as follows:

ALB- Forward :
5-GCCTGCTGACTTGCCTTCATTAG-3
Reverse:
5 TCAGCAGCAGCACGACAGAGTA-3
AFP- Forward:
5-GAAACCCACTGGAGATGAACAGTC-3
Reverse:
5-AAGTGGGATCGATGCAGGA-3
CK-18- Forward:
5-GATCGACCTGGACTCCATGAGAA-3
Reverse:
5-CCGTTGAGCTGCTCCATCTGTA-3

Histological Evaluation

In the end of 3D culture, tissue construct containing hepatocytes like cell were fixed in Bouin's fixative solution. The construct dehydrated through a series of graded alcohols (30, 50, 70, and 90,100), cleared with xylene, and embedded in paraffin wax. Five to seven- micrometer sections were cut from tissue blocks. The sections then stained with hematoxylin & eosin.

Periodic Acid-Schiff Staining (PAS)

Some culture flask of 2D culture were stained by Periodic Acid-Schiff Staining (PAS) method for determine intracellular glycogen.

Statistical Analysis

Data were statistically analyzed by A SPSS software. The difference between the mean was considered significant when $p < 0.05$.

Results

Flowcytometery

were positive for mesenchymal markers such as CD73, but negative against hematopoietic markers, CD31 (Figure 2).

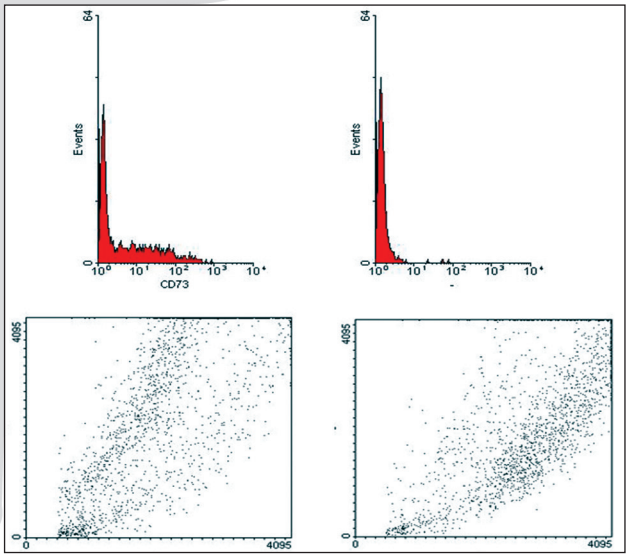

Figure 2. Flow cytometry for MSCs surface markers.CD73 is shown in left graphs and CD3 1is shown in right graphs

Morphology
2D culture - Control group

When qualitative analysis of MSCs morphology was carried out under an inverted microscope,

in normal culture conditions MSCs had normal fibroblastic like morphology. Since there was no stimuli factors for induce hepatic differentiation, rapid proliferation of cells led to cell clusters formation. (Figure 3).

Experimental group

Before the treatment, MSCs did not show any change compared to the controls. In the first step, and after treatment with FGF4, cell proliferation suppressed and elongated fibroblast-like cells were appeared and continuous cluster formation led to decreasing cell size. In second step, HGF-dependent morphological changes such as increased granularity and cell size as well as the increased number of cell nuclei were observed. Cell more obviously changed to flat shape. The cells gradually lost their sharp edges. These changes were more obvious in cluster areas than region with low density whereas cells stopped growing.

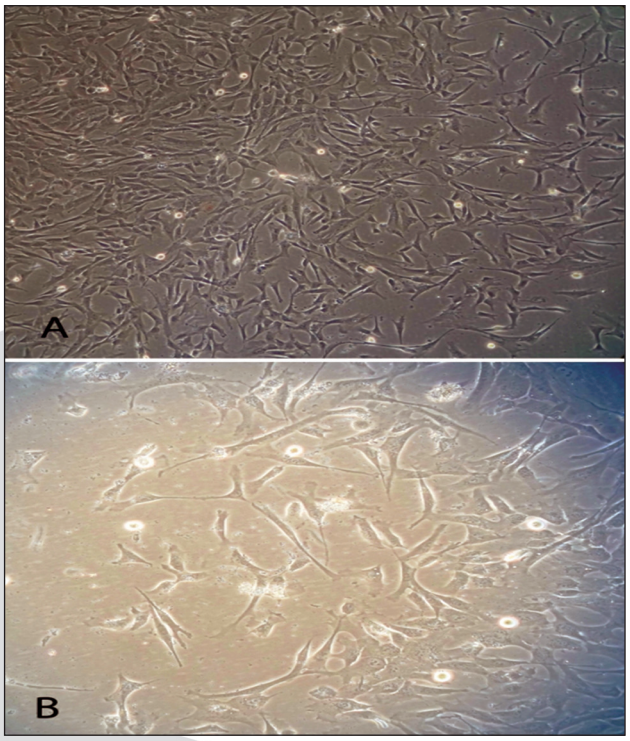

Figure 3. Normal fibroblastic like morphology of MSCs in 2D control group. X100

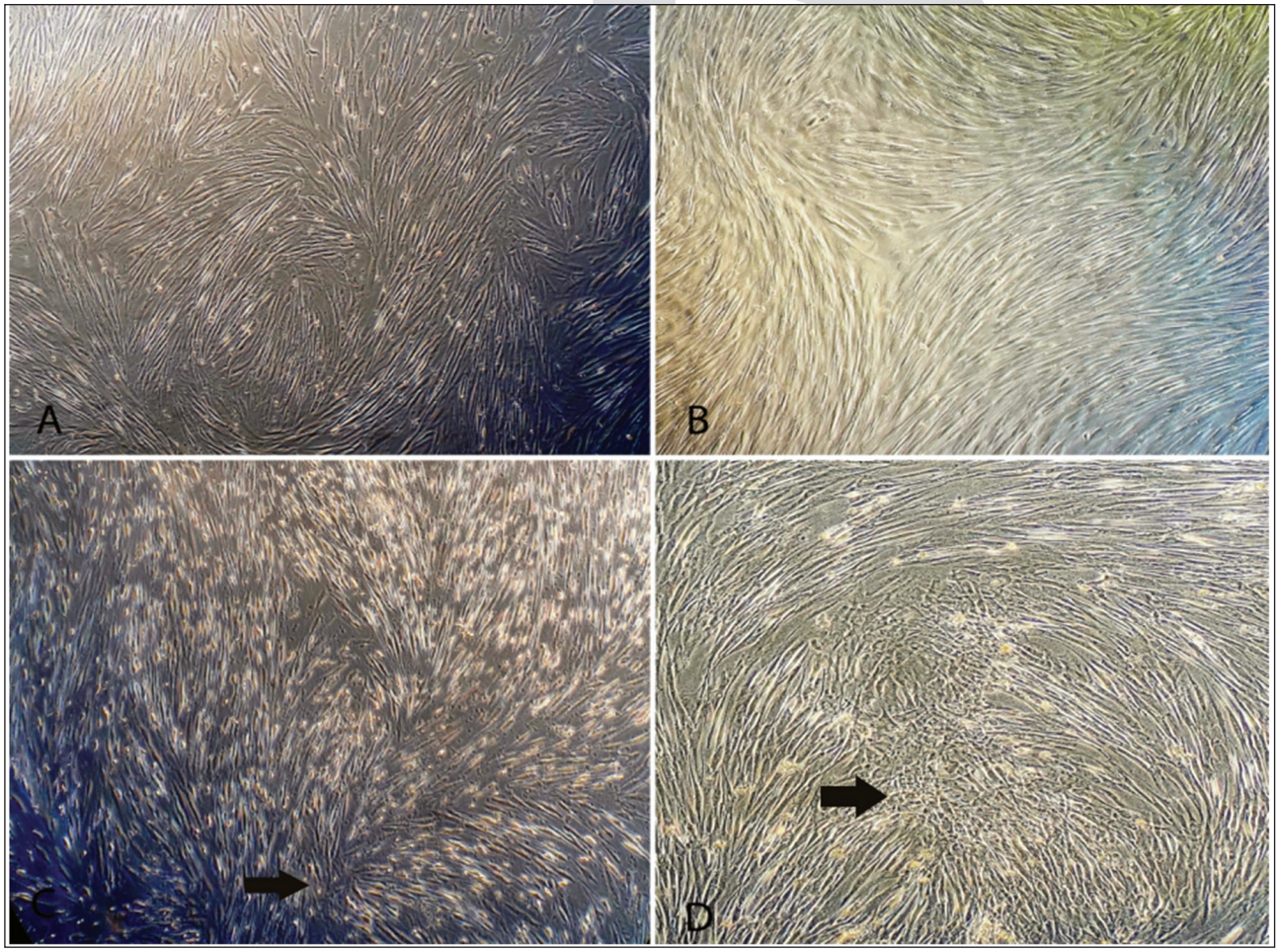

Figure 4. Morphological change of MSCs during four step sequential differentiation. A(step1.), B(step1.), C(step1.), D(step1.). Arrow indicates cell cluster formation. 100x

In the third stage of differentiation, with further exposure to dexamethasone, glucagon, ITS and OSM the morphology cells changed into polygonal-shaped. Granularity and number of cell nuclei increased. In final step, TSA treated cell appeared to possess more granule as well as cell size decreased. Cells displayed rounded epithelial cell morphology (Figures 4, 5, 6).

Three dimensional Culture

Control group:

MSCs showed homogeneous distribution throughout the scaffold and cells had a round-shaped morphology with clear cytoplasm and nucleus on the alginate scaffold. No spread or fused cells were observed and cell kept a round morphology throughout culture time. Since there was no stimuli factors for induce hepatic differentiation, these cells proliferated continually (Figure 7).

Experimental group

After treatment with FGF4, proliferation rates of MSCs encapsulated in alginate are increased. Cells were close together to form cluster. In further exposure to HGF, dexamethasone, glucagon, ITS and OSM several cell clusters of variable size were observed and gradually increased. Alginate beads were filled by cells. In final step, TSA treated cell appeared to possess more compact cluster (Figures 8, 9).

Periodic Acid-Schiff Staining (PAS)

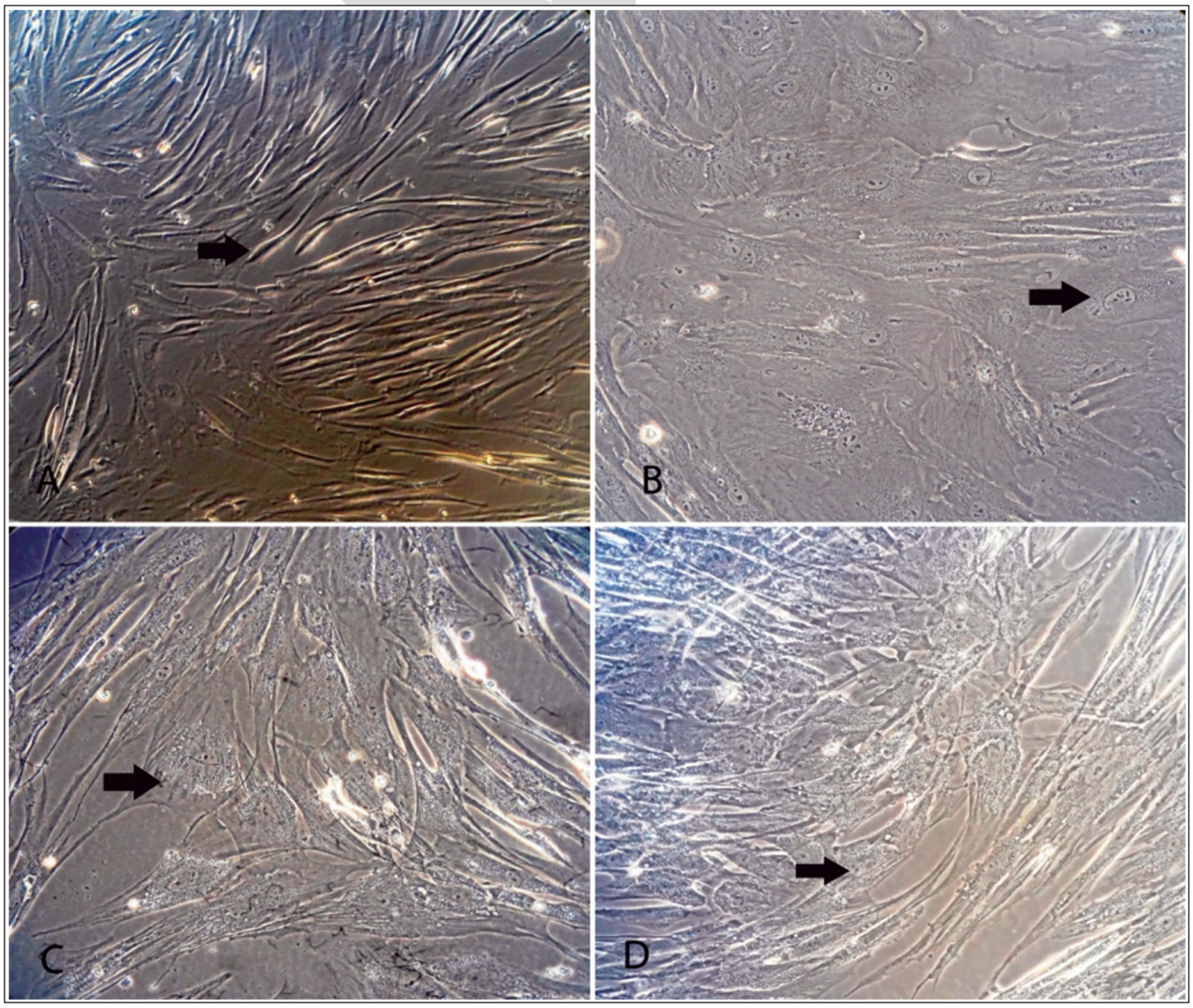

Figure 5. Morphological change of MSCs during four step sequential differentiation. A(step1.), B(step1.), C(step1.), D(step1.). Arrow indicates changes of single cell. 400 X

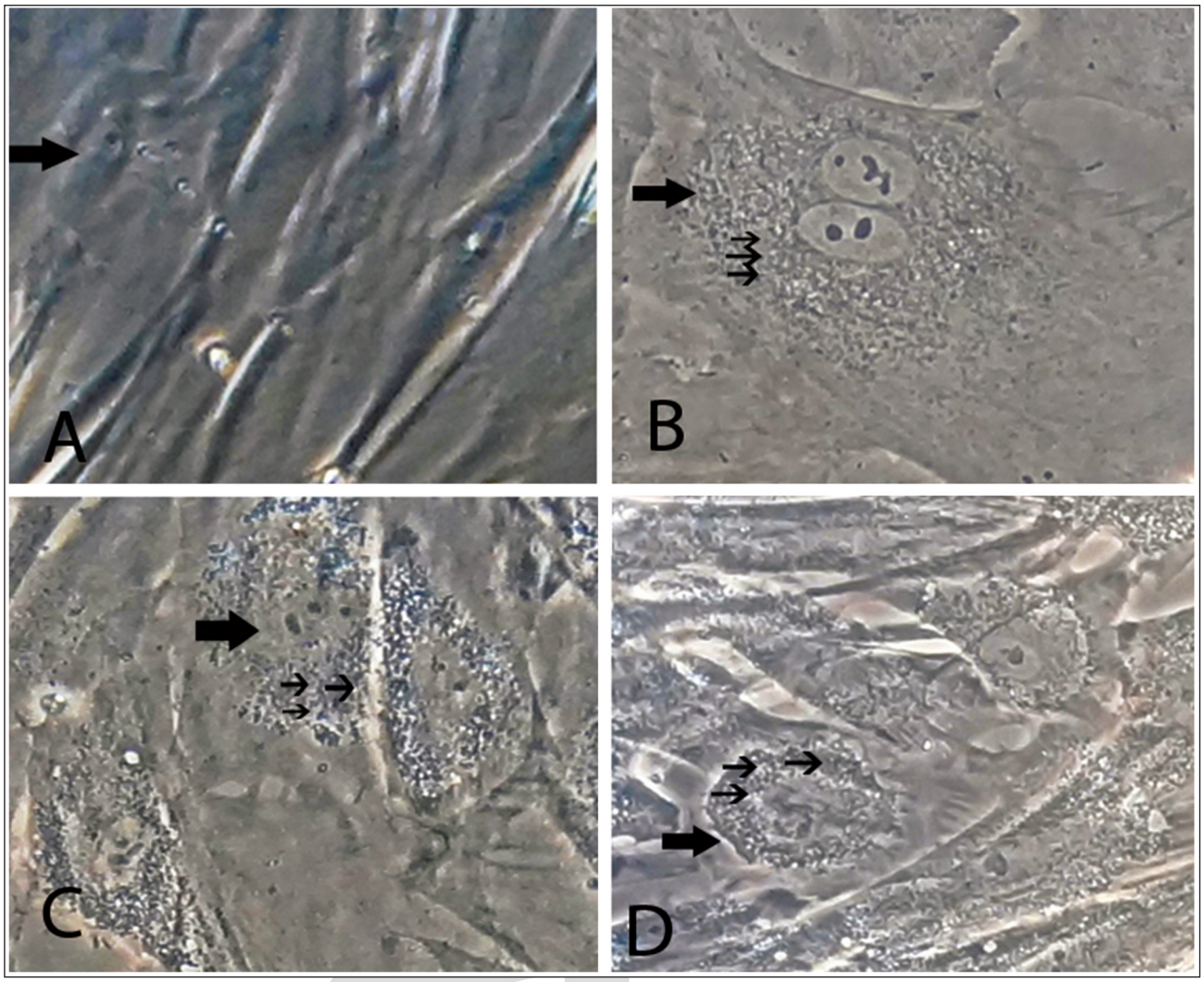

Figure 6. Morphological change of MSCs during four step sequential differentiation. A(step1.), B(step1.), C(step1.), D(step1.). Large arrows indicate changes of single cell. Small arrow indicate cell granules. Some cells revealed bi nucleus morphology.400 X

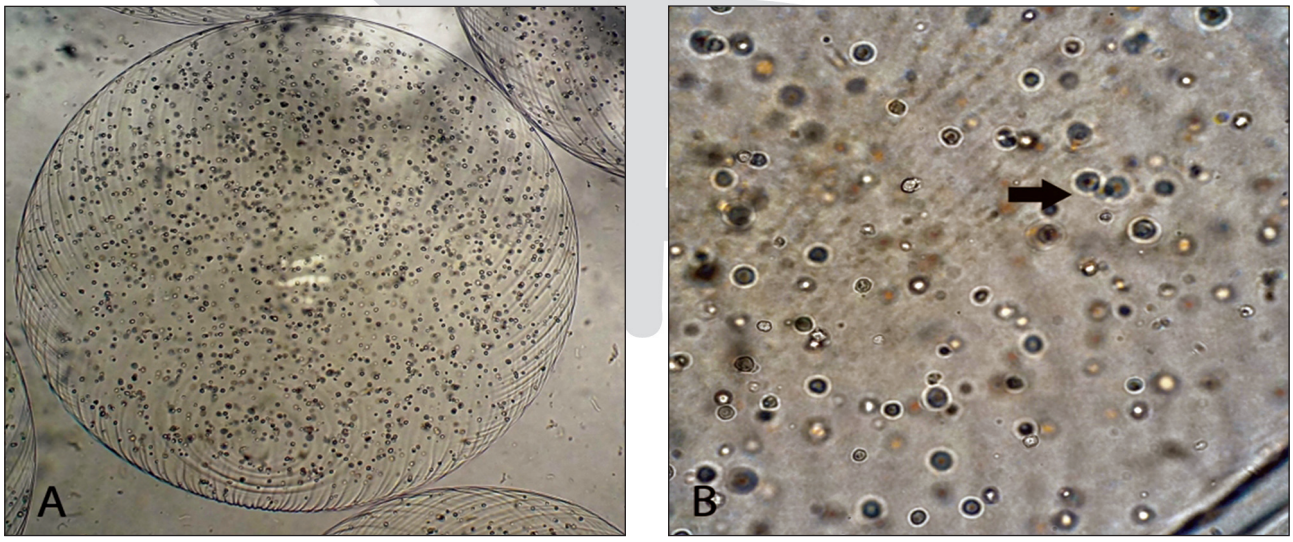

Figure 7. Invert microscopic view of 3D control group. Arrow indicate round morphology of single MSC. A 50x. B 100x

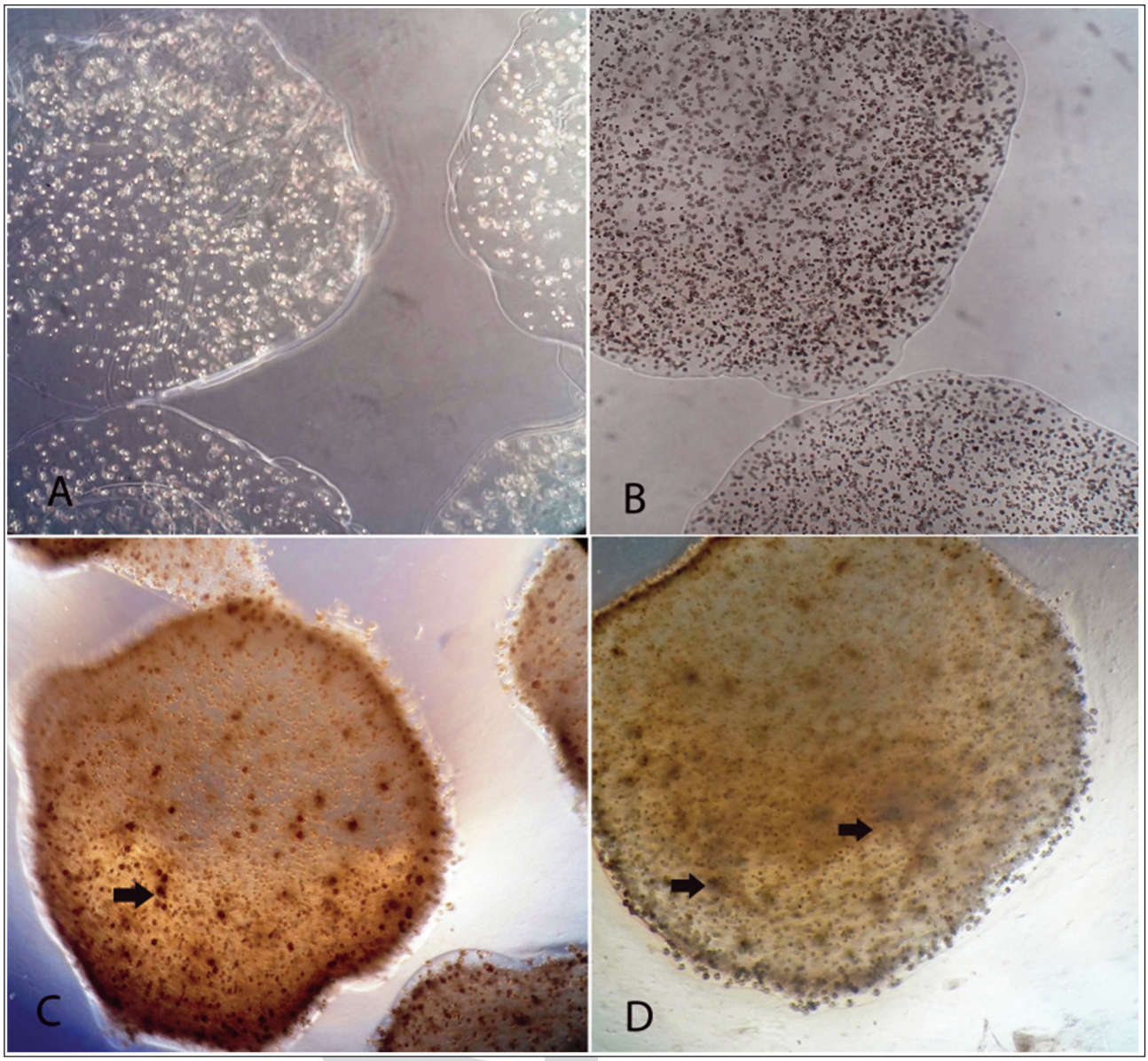

Figure 8. Morphological change of MSCs during four step sequential differentiation. A(step1.), B(step1.), C(step1.), D(step1.). Arrow indicates cell cluster formation. 100x

After staining of differentiated cells in 2D culture by using PAS method, intracytoplasmic glycogen was observed as pink granules and cell nuclei appeared blue by hematoxylin staining (Figure 10).

Histological analysis

Histological examination of samples with hematoxylin and eosin staining revealed clusters of cells with clear cytoplasm and nuclei. Sections revealed feature of multicellular tissue construct. The cells were polyhedral with clear cytoplasm; the cytoplasm were moderately to deeply basophilic, the euchromatin nuclei were observed, the cells often were round and lost their spindle shape. Basically cells were different sizes because of how their differentiation was progressed. Several intracellular clear zones were observed in cells. The cells gradually exhibited morphological changes from a fibroblast-like cell shape in culture flask to hepatocyte-like cells as well as the number of cell-nuclei increased. None of these results were observed in the control group. At first the hepatocyte-like cells resembled cell cluster. Then they arranged in hepatocyte plates cell architecture similar partly similar to normal liver. When examined with higher magnification, several cytoplasmic extension were seen from cells margins. Clear zone were observed in periphery

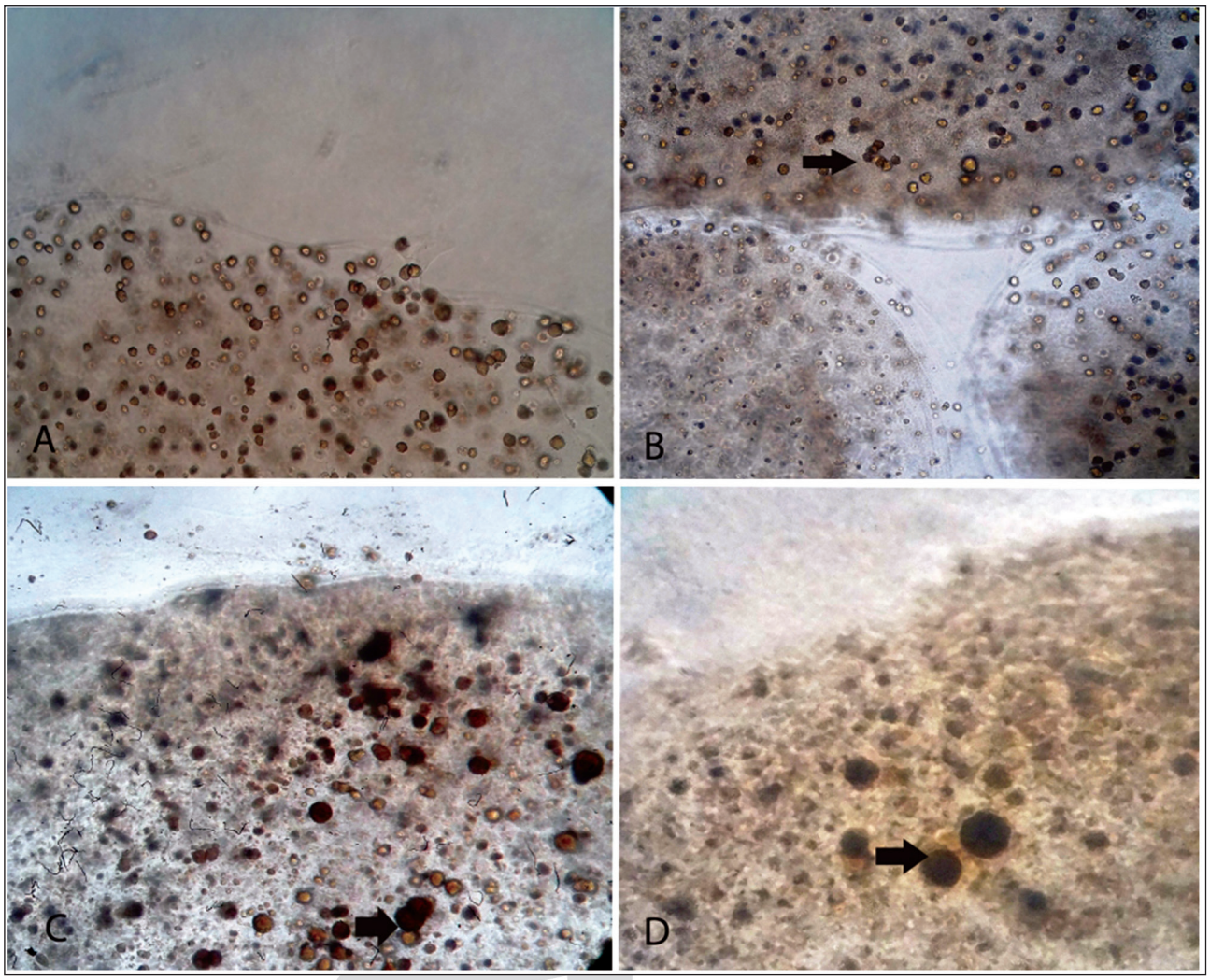

Figure 9. Morphological change of MSCs during four step sequential differentiation. A(step1.), B(step1.), C(step1.), D(step1.). Arrow indicates cell cluster formation. 400x

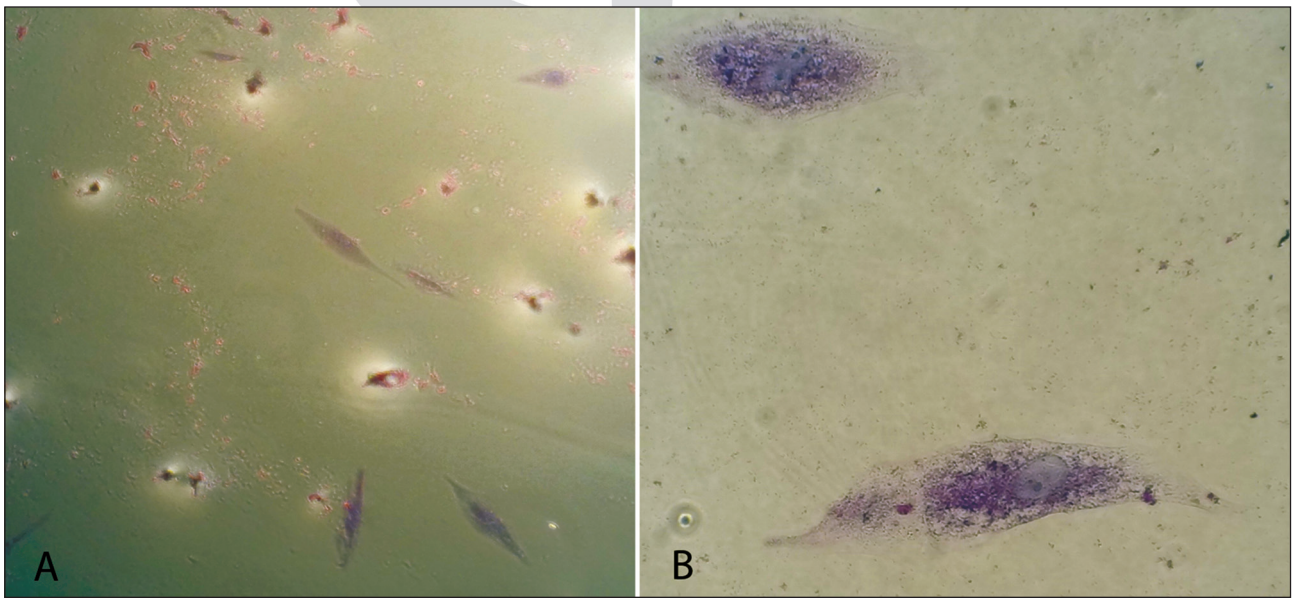

Figure 10. Representative image of PAS staining of MSCs in monolayer culture indicated ability of differentiated MSCs to store glycogen (400x magnification)

of cell. Also several vacuoles were observed at the periphery in the clear zone area where cells were housed in lacuna like spaces. Cells showed homogeneous distribution throughout the scaffold and only a few cells migrated towards the periphery of the beads (Figures 11, 12).

Albumin production

In three-dimensional culture, four days after treatment of cells with FGF4 and HGF, Albumin production activity was observed in differentiated cells. With further exposure to dexamethasone, glucagon, ITS and OSM, albumin production ra-

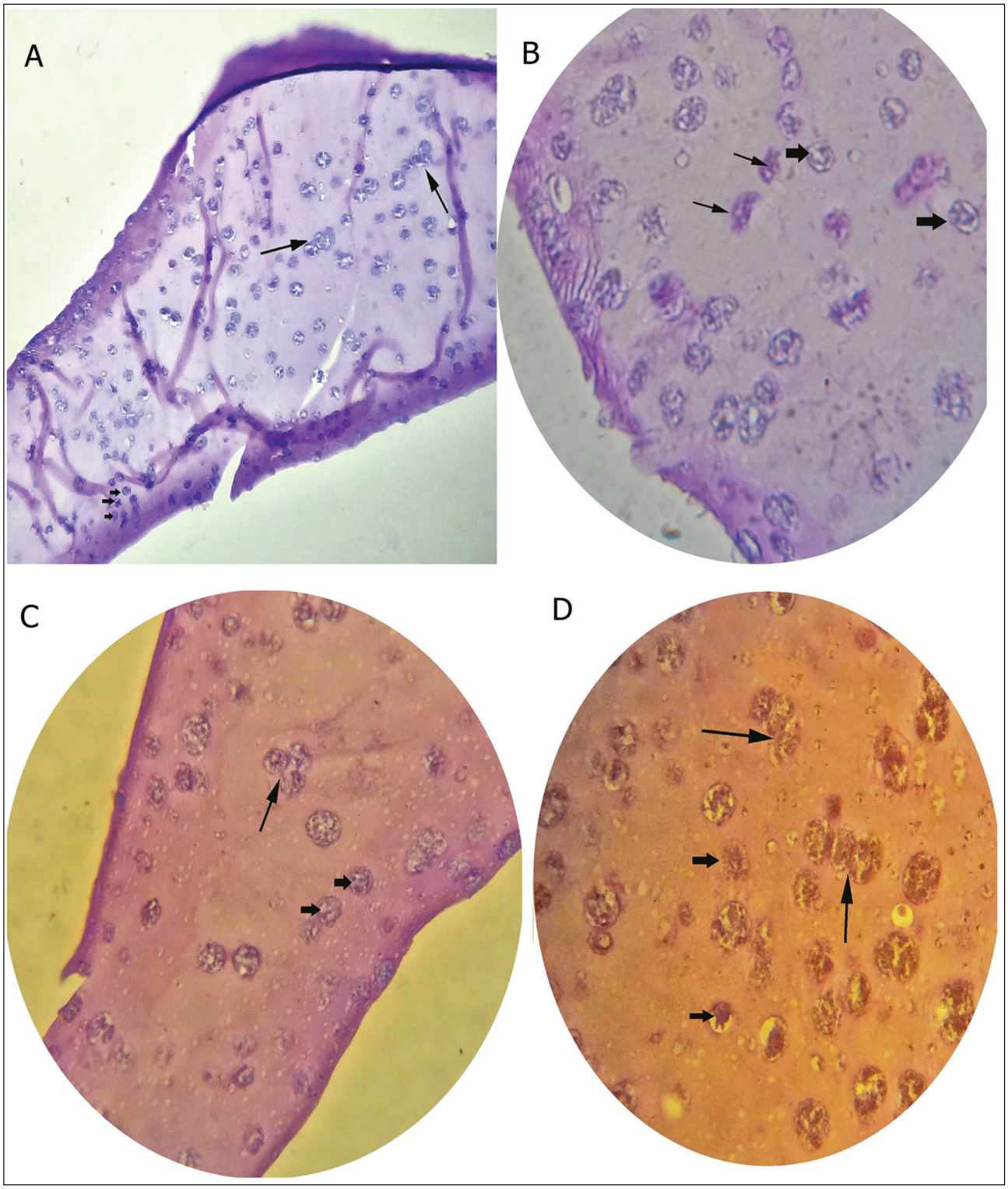

Figure 11. Microscopic view of tissue construct stained by hematoxylin & eosin. A (40x) B-D (200x). arrow indicate cell cluster. Formation of hepatocyte like plate shown in D

tes increased and reached a maximum at day 12. However treatment of cells with TSA in final step, led to decrease albumin production. There was a significant difference between control and experimental groups when ANOVA test was performed to compare the means (p = 0.005) (Figure 12).

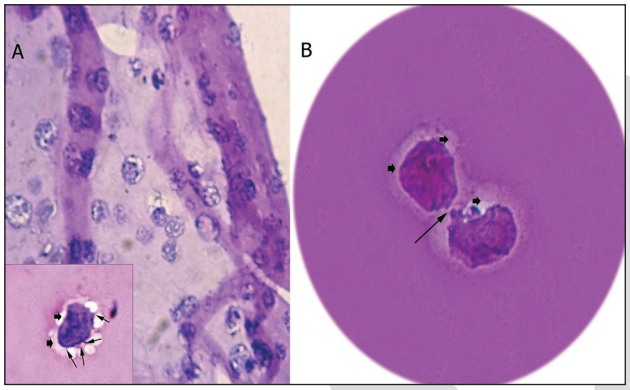

Figure 12. Microscopic view of tissue construct stained by hematoxylin & eosin. In A clear zone and cytoplasmic extension is shown (400x). Clear zone (small) and cytoplasmic extension (long arrow) to formation cell junction is in B (400x)

In two-dimensional culture, 8 days after treatment of cells FGF4, HGF, ,dexamethasone, glucagon, ITS and OSM, Albumin production activity was observed in differentiated cells Albumin production rates decreased over time. After treatment of cells with TSA again at day 14 albumin production increased. There was no significant difference between control and experimental.The mean of albumin production was significantly higher in the experimental group of three-dimensional culture when compared with two-dimensional culture (Figure 13).

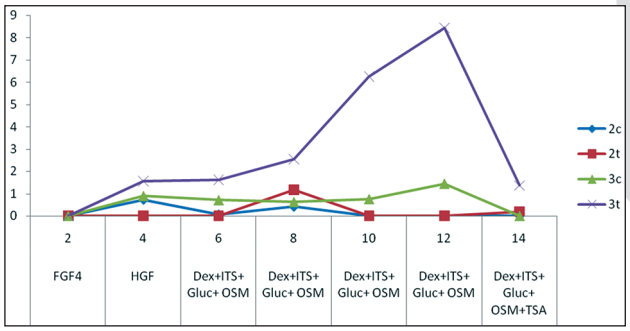

Figure 13. ALB production by MSCs cultured in alginate scaffolds and monolayer culture during differentiation

Urea secretion

Amount of urea secretion by differentiated cells are shown in Figure 14.

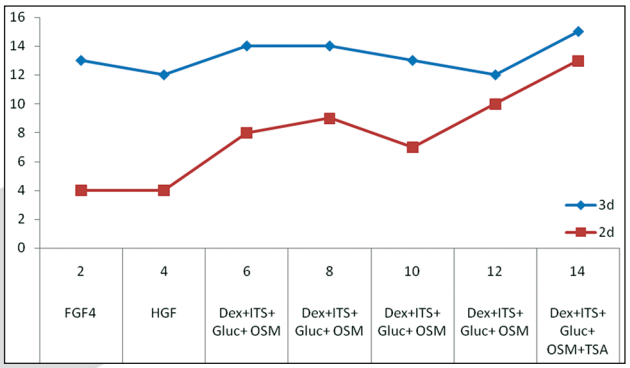

Figure 14. Urea production by MSCs cultured in alginate scaffolds and monolayer culture during differentiation

Three-dimensional culture

Two days after treatment of cells by FGF4 in, secretory activity was observed in differentiated cells. With further exposure to HGF, urea secretion decreased slightly. In third step of differentiation, dexamethasone, glucagon, ITS and OSM led to increase of urea secretion. After treatment of cells with TSA urea secretion reached a maximum at day 14.

Two-dimensional culture

Two days after treatment of cells by FGF4, secretory activity was observed in differentiated cells. Similar to three dimensional culture, dexamethasone, glucagon, ITS and OSM led to increase of urea secretion. After treatment of cells with TSA urea secretion reached a maximum at day 14 (Figure 14).

There was a time dependent increase of urea secretion in experimental groups. Also the mean of urea secretion was significantly higher in the experimental group of three-dimensional culture when compared with two-dimensional culture (p = 0.001) (Figure 14).

Gene expression

Ck18

There was a strong correlation between the expression CK18 gene level and treatment of cells with TSA in final step of differentiation (p=0.015). Exposure of cells to TSA at day14 led to increase of CK18 expression. Since Increased expression of CK18 was observed after exposure to TSA in

both 2D and 3D culture, there was no significant difference between groups (p = 0.180) (Figure 15).

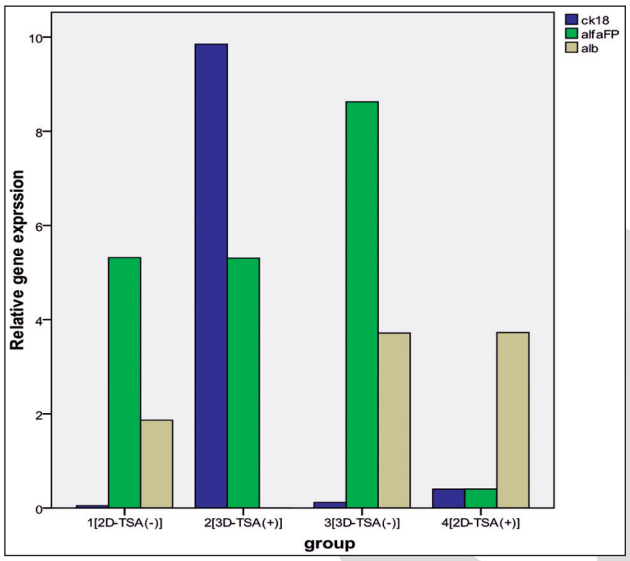

Figure 15. Hepatocyte-specific gene expression determined by RT-PCR

(αFP)

There was a strong correlation between the expression αFP gene level and treatment of cells TSA (p = 0.015). Treatment of cells with TSA at day14 led to decrease of αFP expression (p = 0.026). The results showed a significant difference between expression αFP in both 2D and 3D culture (p = 0.026) (Figure 15).

Albumin

There was a strong correlation between albumin gene expression level and treatment of cells TSA in 3d culture (p = 0.001). Exposure of cells in 3D culture to TSA at day14 led to decreased albumin gene expression. But exposure of cells in 2D culture to TSA at day14 led to increased albumin gene expression, but the increase was not statistically significant (p = 0.083) (Figure 16).

Discussion

In this study, we aimed to investigate the efficiency of a 2D and 3D culture method for in vitro induction of WJ-MSCs into hepatic lineage (Figure 16).

In present study isolated MSCs expressed mesenchymal markers such as CD73, but were negative against hematopoietic markers; CD31. Analysis for immunophenotypic Characterization of MSCs indicated that UC derived cell was one of the MSCs populations similar to those from bone marrow or adipose tissue (7).

In this study, after treatment of cell with FGF4, HGF, dexamethasone, glucagon, ITS, OSM and TSA in 2D culture, morphological changes such as polygonal morphology, increased cellular granularity and appearance of intracyplasmic inclusion bodies were observed. These features were observed by Habibollah (Habibollah, 2010F1F). Also Yoon et al showed that sequential differentiation induced morphological change from spindle fibroblast like to round epithelial-like shape (7).

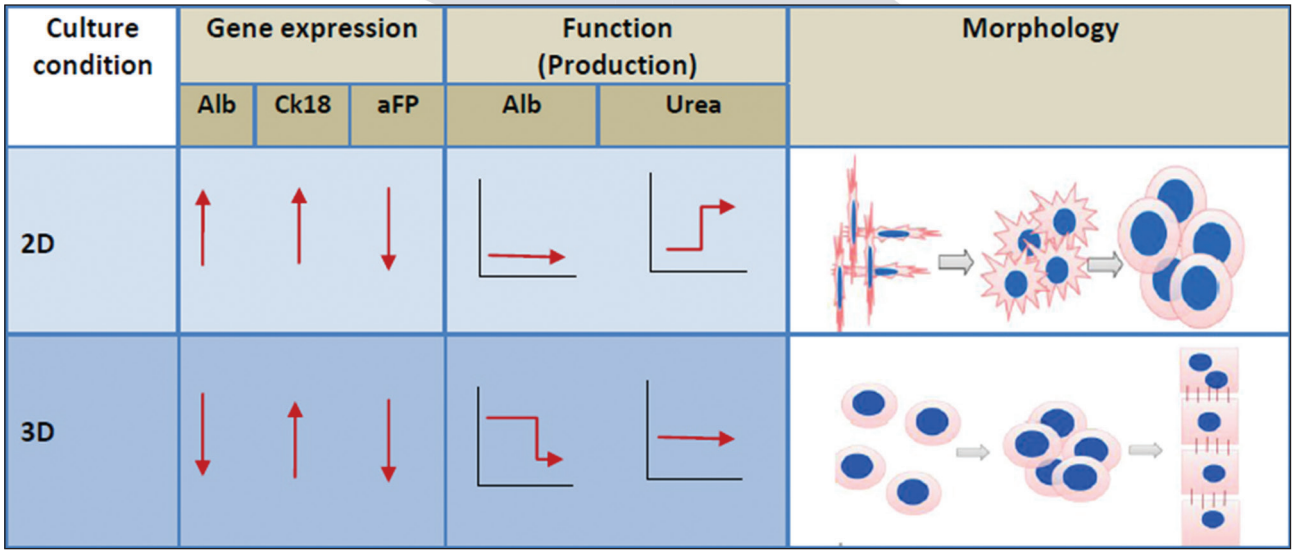

Figure 16. Schematic view of comparison of hepatic differentiation of MSCs in 2D and 3D culture condition

In this study, after treatment of cells with FGF4, HGF, dexamethasone, glucagon, ITS, OSM and TSA in 3D culture, histological examination revealed features of multicellular tissue construct with round shaped cells similar to hepatocyte like cells. Then they arranged in hepatocyte plates cell architecture partly similar to normal liver. Clear zone in periphery of cell was observed. Also several cytoplasmic extensions, vacuoles at the periphery in the clear zone area were observed where cells were housed in lacuna like spaces. Cells showed homogeneous distribution throughout the scaffold and intersingly only a few cells migrated towards the periphery of the beads. Due to limited studies that examined differentiation of mesenchymal stem cell into hepatocyte like cell in alginate scaffold, we couldn't compare our results with others.

Four days after exposure of cells to cytokine and growth factor in 3D culture albumin production activity was observed in differentiated cells. Albumin production reached a maximum at day 12.

However treatment of cells with TSA in final step, led to decrease albumin production. Snykers et al. (2006) reported that TSA plays as proliferation inhibitor and differentiation-inducer agent. In 2D culture, 8 days after treatment of cells with differentiation factors, albumin production activity was observed in differentiated. When compared with 2D culture the mean of albumin production was significantly higher in the 3D culture. In study of Maguire (2005) albumin production was observed in day 8. Also lin (2010) indicated that albumin production is higher in three dimensional condition (Maguire& Davidovich et al., 2007; Lin&Lin, 2010; Snykers&De Kock, 2011).

In 3D culture, 2 days after treatment of cells by FGF4 in, Urea secretory activity was observed in differentiated cells and after treatment of cells with TSA urea secretion reached a maximum at day 14. In 2D culture, 2 days after treatment of cells by FGF4 in, secretory activity was observed in differentiated cells and after treatment of cells with TSA urea secretion reached a maximum at day 14. There was a time dependent increase of urea secretion in experimental groups. When compared the mean of Urea production was significantly higher in the 3D culture. Time of urea production in our study is consistence with Xiao-lei study. Amount of urea production in our study is consistence with Snykers et al.(2005). Maguire et al indicated that TSA lead to increase urea production (5,7).

Exposure of cells to TSA at day14 led to increase of CK18 expression in both 2Dand 3D groups. Exposure of cells to TSA at day14 led to decrease of αFP expression in both 2Dand 3D groups. Exposure of cells to TSA at day14 led to decreased albumin gene expression in 3D culture but increase in 2D culture. Our results is comparable with studies of Zhang, (2009) Kock that RT-PCR analysis demonstrated that UC MSCs expressed the hepatocyte-specific markers ALB, αFP and CK18 following hepatocyte induction (2007)(6,).

Results of the present study indicated that sequential exposure with TSA at the final step suppress albumin gene expression and albumin production activity in differentiated mesenchymal stem cell.

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Left ventricular diastolic dysfunction in atrial fibrillation patients without structural cardiac disease

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Abstract

Background: Hypertension and left ventricular hypertrophy (LVH) are important contributors to left ventricular diastolic dysfunction (DD), left atrial remodeling and atrial fibrillation.

Aims: The principal objective is comparing left atrial (LA) remodeling and left ventricular DD in subgroups of patients with or without paroxysmal atrial fibrillation (PAF) and with or without essential hypertension (HTN).

Material and method: The study included 128 patients with normal systolic function of the left ventricle (LVEF≥50%). They were divided into two groups, according to the presence or absence of essential hypertension. The normotensive patients (n=65) were divided into a PAF group (n=32) and a control no-PAF group (n=33). The hypertensive patients were also divided into a PAF group (n=32) and a control no-PAF group (n=31). The control group patients were chosen to match the PAF groups by sex, age and weight. The patients were evaluated by resting 12 lead ECG, 24 hours Holter ECG monitoring, 24 hours blood pressure monitoring, 2D Doppler echocardiography. The follow up was done at 6 months, for 3 years. Statistical analysis was performed using the MedCalc 12.3.0.0 statistical software for Windows. The results were considered statistically significant when the p-value was <0.05.

Results and conclusion: The LA diameters are larger in PAF patients. HTN leads to supplemental LA increase. The number of PAF episodes does not influence LA diameters. The patients′ gender does not change much the statistical data, with higher diameters in PAF and HTN patients.

Key words: diastolic dysfunction, atrial fibrillation, cardiac disease

Introduction

Atrial fibrillation (AF) is the most common rhythm disturbance, its prevalence increasing by age[1]. In most cases a structural disease can be found, but in a lot of patients (pts) there are no cardiac or extracardiac pathology to be diagnosed. Mitral stenosis, coronary heart disease, hypertension, conditions that lead to left ventricular hypertrophy – are the main cardiovascular contributors to AF etiology.

Hypertrophy and high LV mass are major determinants of diastolic dysfunction (DD). In physiopatological terms, DD means impossibility of left ventricle (LV) to fill itself at low pressure by affecting relaxation and/or filling of one or both ventricles [2]. DD is increasingly recognized as limiting factor of exercise, independent of systolic function which may be normal or impaired [3]. The mechanisms of this dysfunction are not fully understood. In pure DD, diagnosis can be established exclusively by paraclinical means. If DD is associated with clinical signs and symptoms results in diastolic heart failure (DHF) [4], isolated or associated with systolic heart failure.

For diagnoses of isolated DHF three criteria must be present simultaneously: heart failure symptoms and signs, normal systolic function (or slightly impaired) and abnormal relaxation [5] or filling of the LV.

The second criteria is based on echocardiographic measurements (ejection fraction over 45%) and the third criteria can be settle either by echocardiography or cardiac catheterization proving slow isovolumic relaxation and/or early ventricular filling and/or low distensibility of LV and/or increased rigidity of the LV.

Aim and objectives

The principal objective is comparing left atrial remodeling and DD in subgroups of patients with or without paroxysmal AF and hypertension.

The secondary objective is demonstrating the benefit of antihypertensive and antiarrhythmic drugs in decreasing paroxysmal AF.

Material and methods

Patients

The study group included 64 patients with paroxysmal atrial fibrillation and LVEF ≥ 50% admitted in a cardiology clinic, by the following criteria:

- patients with essential HTN and paroxysmal AF (HTN-PAF) as documented by 12 lead ECG or Holter monitoring, with or without symptoms
- patients with no essential HTN but documented paroxysmal AF (nHTN-PAF).

The exclusion criteria were: evident structural heart disease (excluding LVH), moderate or severe mitral regurgitation, aortic regurgitation, coronary heart disease and severe comorbidities.

Patients with severe or secondary HTN were excluded from this study.

The control group consisted of 64 patients without atrial fibrillation. We studied hypertensive patients without paroxysmal atrial fibrillation (HTN-nPAF) as control group for the hypertensive patients with paroxysmal AF (HTN-PAF) and normotensive patients without atrial fibrillation (nHTN-nPAF) as control group for the nHTN-PAF group. The control group patients were selected to match the study groups as closely as possible mainly by age, gender [6], and weight.

The exclusion criteria for the control group were: documented paroxysmal AF (included by programmed stimulation), structural heart disease, moderate or severe mitral regurgitation, coronary heart disease and severe co-morbidities. We also excluded normotensive subjects with echocardiographic LVH signs.

Procedures

Positive diagnosis in paroxysmal AF and HTN (clinical, ECG, Holter monitoring)

Sudden palpitations in a previously healthy patient were the main complaint for referral. This was compounded mainly by vertigo (especially in HTN), decreased effort capacity and unexplainable anxiety.

These patients had a 12 lead ECG as soon as possible. If this rest ECG showed sinus rhythm, we performed Holter monitoring for 24 hours. If Holter ECG was also negative, the patient was not included, but he was instructed to perform ECG recordings whenever symptoms were present. In 75% of these patients, paroxysmal AF could be documented in 30 days.

Blood pressure was determined with the same device, with mercury column, monthly calibrated.

In known hypertensive patients on medication, a detailed history was taken, recording the duration, stage, maximum BP and drug use. The newly diagnosed persons (over 140/90 mmHg), were Holter monitored for 24 h, being diagnosed as hypertensive if BP was over 140/90 mmHg over 25% of the time. The two categories of HTN subjects were included in the study, the treatment was unchanged in known hypertensive subjects, while the newly diagnosed received treatment according to the HTN guidelines. In every person, we measured topometric data for BMI determination, as well as serum creatinin (Cr), urea (U), hemoglobin (Hb), transaminase (ALAT, ASAT). During this initial visit, we excluded structural disease by history, clinic examination, EKG, echocardiography and radiology. In suspected coronary heart disease, effort testing was performed, with angiocoronarography, in positive cases.

Echocardiography parameters

We studied the following parameters:

1. LA dimensions – maximal, endsistolic, before mitral valve opening
   a. LA length – parasternal long axis M mode – Lad (Figure 1)
   b. LA transversal length – apical, 4 chamber 2D – Lat (Figure 2)
   c. LA longitudinal – apical, 4 chamber 2D – Lal (Figure 2)
2. LA surface – apical, 4 chamber 2D - Las
3. Maximum E and A wave velocity - pulsed Doppler at MV apex
4. E/A ratio
5. E wave deceleration time – TDE (Table 1; Table 2).

For subjects with conflicting data, more criteria were used: the Valsalva maneuver and A wave duration in pulmonary veins.

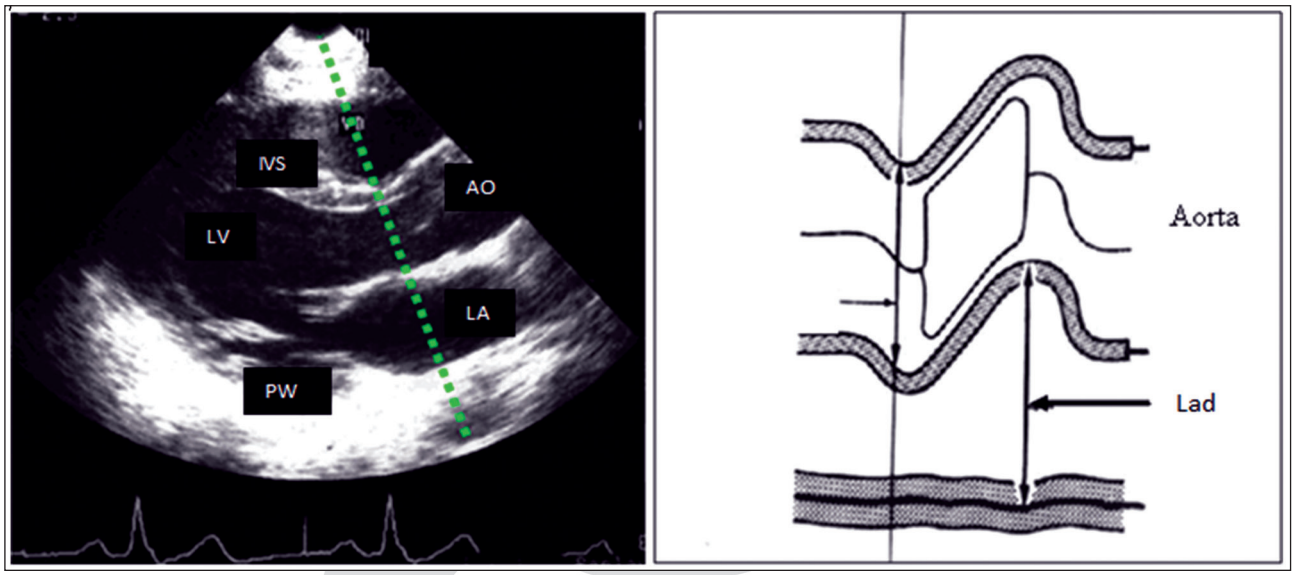

Figure 1. LA length – parasternal long axis M mode – Lad

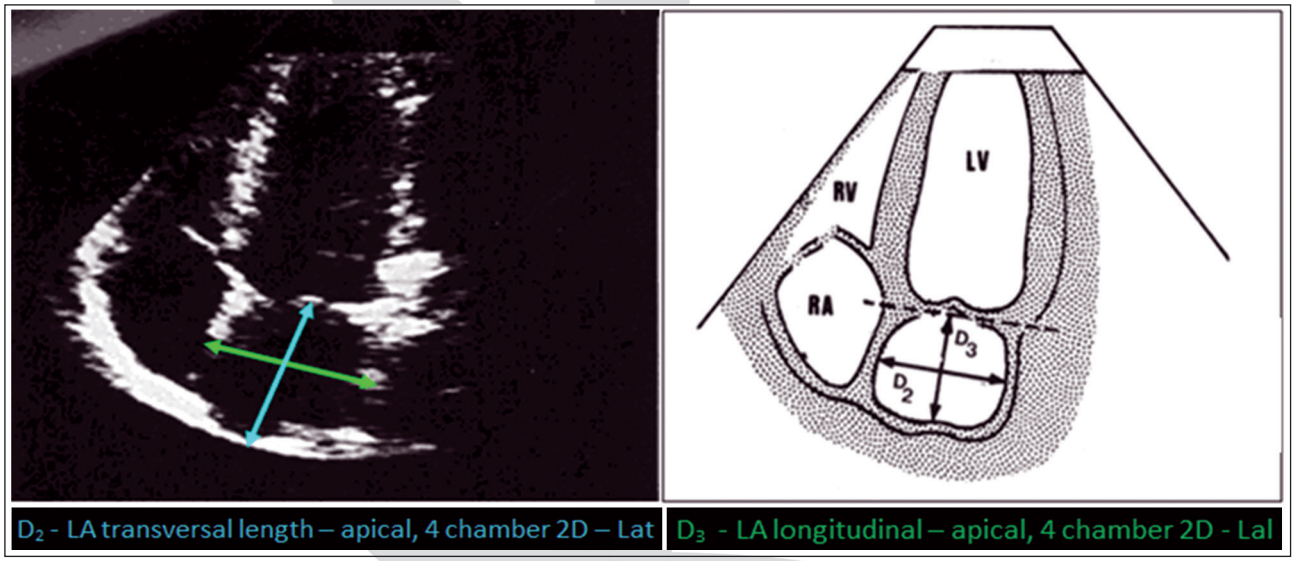

Figure 2. LA transversal and longitudinal length – apical, 4 chamber 2D

Table 1. Diastolic function of the LV - pulsed Doppler at mitral valve apex

Parameters	Relaxation deficit	Normal	Restrictive type
TDE (ms)	> 240	160 - 240	< 160
E m/s	↓	0.8 – 1.5	↑
A m/s	↑	0.75	↓
E/A ratio	E < A	E > A	E >> A
IVRT (ms)	↑	55 - 90	< 70

ms = milliseconds, IVRT = isovolumic relaxation time

Table 2. Different types of diastolic dysfunction

	Diastolic function	Diastolic dysfunction		
	Normal	1	2	3
TDE (ms)	160-240	>240	160-200	<160
E/A ratio	1-2	<1	1-1.5	>1.5
IVRT (ms)	70-90	>90	<90	<70

ms = milliseconds, IVRT = isovolumic relaxation time

Study of blood pressure

The subjects had a 3 years follow up of BP, medication type and treatment efficiency. In known patients we noted the maximal BP as recorded by qualified personal in the last 3 months. After inclusion, BP was measured once/month in the general practice and two/month in our clinic. This was needed to follow a correlation between BP and the AF episodes.

Clinical and paraclinic follow-up

The PAF patients had echocardiography as closely as possible to the sinus rhythm spontaneous conversion. In hypertensive patients, BP measurement was performed simultaneously with echocardiography.

We followed our patients at 6 months for 3 years, with history, clinical examination, and echocardiography. We noted the frequency, rate, duration and symptoms of paroxysmal AF and we monitored BP values and treatment. The paroxysmal AF episodes were documented in the emergency room by the house officer. In this follow period, the control group remained healthy (nHTN and nPAF), the HTN control group has no PAF, the HTN subjects in the first group had paroxysmal AF and the nHTN subjects in the second group had at least one episode of paroxysmal AF - so statistical analysis could be performed correctly.

Results

We studied 128 patients, from which 65 (50,78%) without HTN and 63 (49,21%) with HTN. PAF was present in 64(50%) from the total numbers. The normotensive group was subdivided in two: the control group without PAF (nHTN-nPAF group), n=33 (50,76%) and the group with PAF (nHTN-PAF group) n=32 (49,23%). The control group included 21 female (63,63%) and 10 obese patients. The PAF group included 18 female (56,25%) and 9 obese patients. There was no significant difference between the two normotensive groups obese regarding population characters.

The hypertensive group was also divided into two: the control group without PAF (HTN-nPAF group), n=31 (49,20%) and the group with PAF (HTN-PAF group), n= 32 (50,79%). The HTN-nPAF group included 17 female (54,83%) and 17 obese patients. The HTN- PAF group included 18 female (56,25%) and 20 obese patients (62,50%). There was no significant difference between these groups by regarding population characters.

Subgroup study

Echografic measurements compared the variables (LA diameters and surface, E/A ratio, TDE) as follows: nHTN-nPAF versus nHTN- PAF group, HTN-nPAF versus HTN-PAF group and nHTN-PAF versus HTN - PAF group.

nHTN-nPAF versus nHTN- PAF group

LA diameters achieved statistical significance – Lad (p=0.0001), Lal (p=0.0001), Lat (p=0,0001) – demonstrating that in PAF persons these diameters are larger than in normal subjects. The LA surface achieved high significance, being larger than in the normal group. The E/A ratio was significant (p=0,0001) while the TDE value was null.

HTN-nPAF versus HTN- PAF group

The results are statistically significant- Lad (p=0,0001), Lal (p=0,0001), Lat (p=0,0001) – proving that in PAF hypertensive persons, LA diameters are larger. The LA surface is significantly greater in HTN-PAF persons. The diastolic function was influenced significantly in the E/A ratio (p=0,01), but not in TDE.

There were no significantly differences in BP, so we concluded that PAF was the cause of LA dilation.

nHTN-PAF versus HTN - PAF group

To see if HTN is an independent risk factor for DD, we evaluated LA parameters in two groups of patients with PAF, comparable by age (p=0,61), weight (p=0,36), height (p=0,51) and BMI (p=0,1).

Results are statistically significant – Lad (p=0,002), Lal (p=0,0001), Lat (p=0,0003) - with higher LA diameters and surface in HTN-PAF patients. The E/A ratio was also significant (p=0,037), while TDE was not. The diameters surface and E/A ratio gradually, HTN group, proving that PAF leads to LA dilation and HTN increases further this dilation.

The LA dimensions increase in all directions with consecutive surface increment.

Gender related subgroup studies

We compared the above mentioned variables between the sexes, with the same subgroup and between the study group.

nHTN-nPAF versus nHTN- PAF group

Normal group nHTN-nPAF had 33 patients: 21 (63,63%) patients were females, which was named nHTN-nPAF$_F$. The nHTN-PAF group had 32 patients: 18 patients (56,25%) were females which was named nHTN-PAF$_F$. The two subgroups were comparable by age (p=0,055), weight (p=0,513), height (p=0,597) and BMI (p=0,759). The LA diameters, surface, E/A ratio were statistical significance, while TDE was not - Lad (p<0,001), Lal (p<0,0001), Lat (p<0,001), Las (p=0,002), E/A (p<0,001).

Normal group nHTN-nPAF had 33 patients, 12 patients (36,36%) were men which was names nHTN-nPAF$_M$ and the nHTN -PAF group had 32 patients, 14 patients (43,75%) were men, which was named nHTN -PAF$_M$. The two subgroups were comparable by age (p=0,488), weight (p=0,789), height (p=0,736) and BMI (p=0,835). The LA diameters, surface, E/A ratio were statistical significance, while TDE was not - Lad (p=0,008), Lal (p<0,0001), Lat (p=0,049), Las (p=0,007), E/A (p=0,004).

In both, men and women, diameters, surface and E/A ratio had statistical significance, while TDE hadn't. In the men subgroup, Lat nearly lost statistical significance with p=0,049.

In conclusion, LA dilation and DD are significantly greater in PAF group than in normal, regardless of the patients' gender.

HTN-nPAF versus HTN- PAF group

HTN-nPAF group had 31 patients, 17 (54,83%) patients were females, which was named HTN-nPAF$_F$, and the HTN-PAF group had 32 patients, 18 patients (56,25%) were females which was named HTN-PAF$_F$.. The two subgroups were comparable by age (p=0,062), weight (p=0,166) and BMI (p=0,993). Height had a p of 0.018 but the BMI remains not significant. There were no significant differences between systolic and diastolic blood pressure. The LA diameters, surface were statistical significance, while E/A ratio and TDE were not - Lad (p=0,004), Lal (p=0,006), Lat (p=0,02), Las (p<0,001).

HTN-nPAF group had 31 patients, 14 patients (45,16%) were men which was named HTN-nPAF$_M$. The HTN-PAF group had 32 patients, 14 patients (43,75%) were men, which was named HTN-PAF$_M$. The two subgroups were comparable by age (p=0,795), weight (p=0,983), height (p=0,577) and BMI (p=0,690).

Only one of the LA diameters achieved statistical significance, Lat (p=0,047) and the LA surface (p=0,004), as well as the E/A ratio (p=0,006), while TDE did not change. In conclusion, LA dilation and DD are significantly greater in the HTN-PAF group regardless of the patients' gender, but some LA diameters do not achieve statistical significance.

nHTN-PAF versus HTN - PAF group

nHTN-PAF group had 32 patients: 18 (56,25%) patients were females, which was named nHTN-PAF$_F$. The HTN-PAF group had 32 patients: 18 patients (56,25%) were females which was named HTN-PAF$_F$. The two subgroups were comparable by age (p=0,470), weight (p=0,441), height (p=0,305) and BMI (p=0,177). Two of LA diameters were statistical significance, while surface, E/A ratio TDE was not - Lad (p<0,014), Lal (p<0,001).

nHTN-PAF group had 32 patients, 14 patients (43.75%) were men which was names nHTN-PAF$_M$ and the PAF-HTN group had 32 patients, 14 patients (43,75%) were men, which was named PAF-HTN$_M$. The two subgroups were comparable by weight (p=0,540), height (p=0,855) and BMI (p=0,378) but age had a significant p (0.037). Two of LA diameters and surface were statistical significance, while E/A ratio and TDE was not - Lal (p<0,0001), Lat (p<0,001) and Las (p=0,014).

In both, men and women, diameters, surface and E/A ratio had statistical significance but some of diameters lost statistical significance; it means that the left atrium increases in some directions more than in others.

In conclusion, LA dilation and DD are significantly greater in PAF group than in normal, regardless of the patients' gender.

Studies by age group

nHTN-nPAF vs nHTN- PAF group

The two age groups were 20-39 years and 40-65 years; in the nHTN-nPAF group, the first age

subgroup had 9 persons and the second had 24 persons. The first nHTN-PAF age group included 9 patients, the second age group included 23 persons.

The first age group was small, but homogenous – weight (p=0,7), height (p=0,617), BMI (p=0,976). One of the LA diameters and E/A ratio had statistical significance - Lal (p<0,001), E/A (p=0,003) -the other diameters surface and TDE were not.. This may promote the significance of longitudinal LA diameter which remains significant in those conditions too (low patients number).

In the second age group – weight (p=0,739), height (p=0,868), BMI (p=0,865) - all diameters, surface and E/A ratio were statistical significance, TDE was not - Lad (p<0,001), Lal (p<0,001), Lat (p<0,001), Las (p<0,001) and E/A (p<0,001).

In conclusion, LA dilation is significant in PAF patients even by age group.

HTN-nPAF versus HTN-PAF group

We formed a 30-45 years group and a 46-60 years group. In the hypertensive group without PAF, 8 patients were in the first subgroup and 23 patients were in the second, while in the hypertensive group with PAF, there were 8, respectively 24 patients in each age group.

In the 30-45 age groups the clinical characteristics are comparable – weight (p=0,476), height (p=0,847), BMI (p=0,640), systolic BP (p=0,376), diastolic BP (p=0,271). LA diameters and surface are significantly larger in PAF, so Lad (p=0,031), Lal (p=0,002), Lat (p<0,01), Las (p=0,007). The E/A ratio and TDE are not significant.

In the 45-65 age groups, the clinical characteristics are also comparable – weight (p=0,4731), height (p=0,296), BMI (p=0,987), systolic BP (p=0,211), diastolic BP (p=0,192). The LA diameters, LA surface and ratio E/A are significantly larger in the HTN- PAF group: Lad (p=0,015), Lal (p=0,025), Lat (p=0,035), Las (p=0,001) and E/A ratio (p=0,017).

In the HTN-PAF group the diameters, surface and E/A ratio are significantly increased compared to HTN-nPAF group, even in the same age group.

nHTN-PAF versus HTN-PAF group

We formed a 20-45 years group and a 46-60 years group. In nHTN-PAF group, 15 patients were in the first subgroup and 17 patients were in the second one, while in the HTN-PAF group, there were 8, respectively 24 patients.

In the 20-45 age groups the characteristics are comparable – weight (p=0,445), height (p=0,40), BMI (p=0,76) - LA diameters and surface are significantly larger in PAF-HTN, so Lad (p=0,01), Lal (p=0,002), Lat (p=0,049), Las (p=0,038). The ratio E/A and TDE are not significant different.

In the 45-65 age groups, the characteristics are comparable – weight (p=0,569), height (p=0,763), BMI (p=0,0,645). Only the diameters – Lad (p=0,0001), Lat (p=0,002) and surface Las (p=0,043) are significantly larger in HTN-PAF patients. Lad, E/A ratio and TDE are not significant different.

The study of diastolic dysfunction

We analyzed the occurrence of DD in different subgroups as well as the DD types. We compared nHTN-nPAF versus nHTN-PAF, HTN-nPAF versus HTN-PAF and *nHTN-PAF versus HTN-PAF group.*

nHTN-nPAF versus nHTN- PAF group

From the 33 patients of the *nHTN-nPAF* group, 27 patients (81,82%) had no DD and 6 patients (18,18%) had DD type I. DD type II was not present in this group.

From the 32 patients of the nHTN- PAF group, 11 patients (34,38%) had no DD, 14 patients (43,75%) had DD type I and 7 patients (21,88%) had DD type II.

The number of DD patients is significantly larger in the PAF group (p=0,0001). We followed up our analysis to see if in DD as a whole, the subtype I and II, remains with statistical significance. Comparison between the patients without DD and the patients with DD type I shows a strong statistical significance (p=0,004). These results confirm that type I DD is more frequent in the PAF group. Comparison with type II DD shows a strong statistical significance, as well, meaning that type II DD is more frequent in the PAF group then in nPAF group. To increase statistic power, we compared the groups without DD, with type I DD and with type II DD (p=0,0002).

Statistical analysis shows that DD is more frequent in PAF then in normal subjects, as a whole and in different DD types.

HTN-nPAF versus HTN-PAF group

From 31 patients of the group HTN-nPAF, 11 patients (35,48%) hadn't DD, 15 patients (48,39%) had DD type I and 5 patients (16,13%) had DD type II.

From 32 patients of the group HTN-PAF, 4 patients (12,50%) hadn't DD, 24 patients (75%) had DD type I and 4 patients (15,50%) had DD type II.

There are more patients with DD in the HTN-PAF group (p=0,004). Comparison between patients with DD type I and without DD shows that there are statistical changes (p=0,033). Results confirm that type I DD is more frequent in patients with HTN-PAF. We compared the two DD subgroup, with no statistical significance.

In conclusion, statistical significance changes appear regarding DD between HTN-nPAF versus HTN-PAF group mainly due to DDI type.

nHTN-PAF versus HTN-PAF group

We statistically analyzed the two DD types, between the nHTN-PAF group and HTN- PAF group and we saw significant change (p=0,034). DD is more frequent in patients from the HTN-PAF group, not exclusively to one DD type but due to both DD types.

The study of medication for HTN and PAF

HTN-PAF patients were divided by their medication: one received a beta-blocker with diuretic (BB+D), while the other one received AT II blocker plus diuretic (AT II+D). From the 32 patients from HTN –PAF group, 14 patients (43,75%) were in the beta-blocker group and in the ATII blocker group were 18 patients (56,25%).

We followed the occurrence of PAF in these patients, to see which medication offers better antiarrhythmic protection. We divided the groups in a single episode group and a multiple episodes group.

We didn't take into account the medication type within the same class, but we tried to administrate the recommended doses.

In the BB group, 3 patients had one episode of PAF, and the rest 11 patients had more than 2 episodes.

The statistical changes are significant, showing that in the AT II+D group, the PAF episodes are less frequent than in the BB+D group.

Conclusions

LA diameters and paroxysmal atrial fibrillation

1. The LA diameters are larger in PAF patients. This remains true in hypertensive patients, where further LA enlargement is due to HTN
2. HTN leads to supplemental LA increase - the patients with HTN and PAF had larger LA diameters comparative with normotensive PAF patients
3. The number of PAF episodes does not influence LA diameters, a greater number of arrhythmic episodes does not lead to greater dilation than a single one
4. The patients′ gender does not change much the statistical data with higher diameters in PAF and HTN patients, although some diameters are less significant. Therefore to demonstrate LA dilation, we must follow those diameters that retain statistical significance, in other words sex change atrial dilation parameters as following:
   - Lat in male patients - in HTN- nPAF versus HTN –PAF group
   - Lad and Lal in female patients – in nHTN-PAF versus HTN –PAF group
   - Lal and Lat in male patients – in nHTN-PAF versus HTN- PAF group
5. Comparison between age groups shows that in the same age group, LA dilation is greater in PAF group versus no PAF group, in normo- and hypertensive patients:
   - in the 20-39 age group, only the Lal diameters are statistical significant in comparing nPAF group versus PAF group
   - in the 46-65 age group, only Lal and Lat diameters are statistical significant in comparing nHTN-PAF versus p HTN-PAF group.

LA diameters and DD

1. Larger LA diameters lead to LV diastolic dysfunction occurrence
2. Diastolic dysfunction is more frequent in PAF patients, regardless of blood pressure

3. PAF and HTN lead to LA dilation and LV diastolic dysfunction, so that correction of those factors may lead to improvement of high end-diastolic left ventricular pressure.

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Vertebrate specific oncogenic TAC1 has unconventional networking properties

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Abstract

Introduction: Substance P (SP) is a brain-gut hormone, the best known member of the tachykinin family, encoded by Tachykinin 1 gene (TAC1). Alterations in the expression of Substance P can lead to many pathophysiological conditions including cancers, migraine and mental disorders. The aim of this study was to obtain information about the network properties, evolution and duplicability of this gene through computational analysis.

Methods: 26 orthologs of TAC1 were found by using the eggNOG database and paralogs by the UCSC genome browser. Transmembrane topology of the SP receptor, Neurokinin 1 (NK1-R) was obtained by using CONPRED II database. Phylogenetic tree was produced by using MAAFT online version 6.0, STRING database version 8.3 was employed for protein-protein interaction network and WEBLOGO version 3.0 was used for amino acid sequence conservation.

Results: This analysis suggests that TAC1 appeared in vertebrates which implicates TAC1 in complex processes and is a new gene. Amino acid sequence conservation analysis suggests its high conservation.

Conclusion: Being singleton suggests that this gene is involved in vital physiological functions in many organisms including humans and is prone to induce multiple pathologies. Mutated TAC1 can result in brain and gastrointestinal disorders. TAC1 gene is involved in many cancers but unlike other cancer genes it has a small network and further studies are needed to discover other interactors. Computational analysis reveals that the SP encoding gene TAC1 is very important functionally and a mutation or alteration in this gene can lead to a terminal disease. It also suggests that TAC1 controls many important pathways in human nervous system regulation.

Key Words: Substance P, computational analysis, protein-protein interaction, Tachykinin 1 gene

Introduction

The Tachykinin (TK) family of neuropeptides is one of the largest peptide families described in animals. More than forty tachykinin (TK) peptides have been identified in the animal kingdom including invertebrates, protochordates and vertebrates[1]. Substance P (SP), Neurokinin A (NKA) and Neurokinin B (NKB) are the best known members of this family[2] in mammals. TKs are evolutionary conserved excitatory neuropeptides or peptide hormones characterised by a common, specific C-terminal signature motif: FXGLMNH2. X is an aromatic or aliphatic amino acid. This hydrophobic and amidated sequence is important for specific responses and generation of signal pathways; it is also necessary for TK receptor binding.

Three genes namely TAC1, TAC3 and TAC4, belonging to the family of preprotachykinins (PPTs), encode TKs[3]. Previously these genes were called PPT-A, PPT-B and PPT-C respectively[4]. PPTs are larger precursor molecules that produce many peptides after differential RNA splicing. Mammalian Substance P is derived from the preprotachykinin-A (PPT-A) or TAC1 gene (Figure 1), which originates from a common ancestral gene by duplication[5].

The tachykinin peptides Substance P (SP), neurokinin A (NKA), neurokinin A (3-10), neuropeptide K (NPK), and neuropeptide gamma (NPγ) are produced from a single preprotachykinin gene PPT-A or TAC1 as a result of differential RNA splicing (Figure 1) and differential posttranslational process-

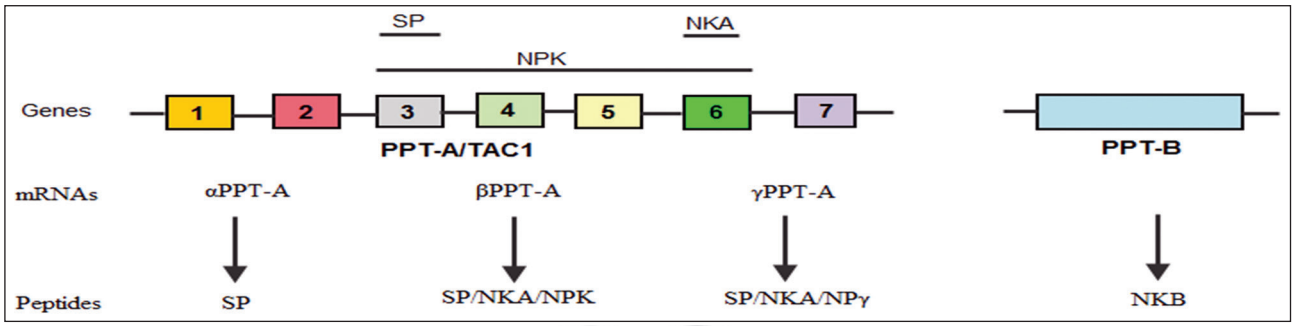

Figure 1. Structure of TAC1 gene[6]

Table 1. Tachykinin receptors 1 Gene

TK receptor	Affinity for TKs	Location	Amino acids	Genes	Gene ID	Chromosome location	mRNA ID	Protein ID
NK-1R	SP>NKA>NKB	CNS/PNS	407	TACR1	6869	2p13.1-p12	NM_001058	NP_001049

ing[7]. PPT-A/TAC1 comprises of seven exons, which can be alternately spliced and modified to form four transcripts: α, β, γ, and δ (Figure 1). SP is encoded by exon 3, present in each transcript, and NKA is encoded by exon 6, present only in transcripts β and γ [3]. NPK is produced by β*TAC*1 and γNPbyγPPT-A/γ*TAC*1[8, 9] by differential processing at the N-terminal dibasic cleavage site of NKA[10]. The mRNA of each uses a different exon corresponding to the protein coding region. αPPT-A contains all the exons except 6 while exon 4 is missing in γPPT-A mRNA. βPPT-A mRNA comprises of all 7 exons (Figure 1)[6]. α PPT-A mRNA expression is more abundant in the brain, while βPPT-A and γPPT-A mRNAs are predominately expressed in peripheral tissues[11].

Preprotachykinin contains a signal peptide of 16-30 residues at N-terminal, one or several copies of a neuropeptide and one or more spacer parts and is formed after the translation of mRNA from PPT-A, PPT-B or PPT-C in the nucleus. It is then transported to the endoplasmic reticulum where it is converted to propeptide after the removal of signal peptide. From here this propeptide is transported to the Golgi complex where the spacer parts are clipped off to yield the final active peptide sequence[12]. These active peptides are then packed into secretory granules, leave the golgi apparatus and are then transported through the axon to the nerve terminals[13].

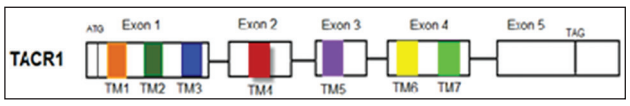

Figure 2. Tachykinin1 receptor gene,

All the physiological responses of TAC1 gene are mediated by Neurokinin receptor 1 (NK-1R) which binds preferentially to SP (Table 1). HK-1, EKA and EKB also prefer NK-1R[14]. NK-1R belong to family 1 (Rhodopsin like) hydrophobic7-transmembrane (TM1-TM7), G protein coupled receptors (GPCRs)[15]. It has 3 extracellular and 3 intracellular loops, one extracellular amino terminus and one intracellular carboxy terminus[16] (Figure 3). It is involved in many biological processes[17].

Tachykinin neuropeptide, SP brings all of its cellular activities after binding to the G-protein coupled receptor NK-1R which is located on the cell surface[7]. Like all TK receptors, SP also binds to heterotrimeric G-protein complex with a preference for Gs and Gq. Binding of the receptor to Gs stimulates adenylyl cyclase and cyclic AMP production while binding to Gq initiates the phosphatidyl-inositol cascade[18]. Upon binding of SP to NK-1R, a signal transduction cascade is initiated by internalization of SP-NK-1R complex[19] resulting in activation of Phospholipase C (PLC). PLC is a second messenger and produces inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG)[20]. IP3 stimulates the endoplasmic reticulum to release intracellular calcium and initiates many signal transduction cascades. DAG activates protein kinase C (PKC)[21, 22].

The present study was conducted to understand the network properties, evolution and duplicability of the gene encoding Substance P (Tachykinin 1) by computational analysis.

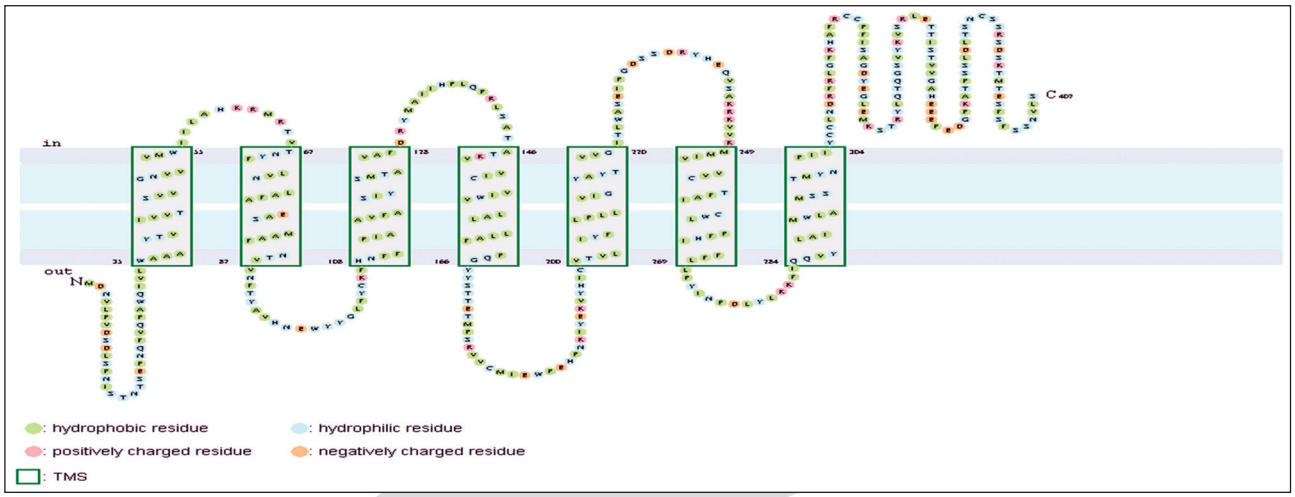

Figure 3. Transmembrane topology prediction of NK-1R by using ConPred II

2. Data mining for TAC1

System level properties such as genomic duplication, network properties and phylogenetic analysis of TAC1 gene were performed. Eggnog database Version 2.0 (http: //eggnog.embl.de) was used to locate the orthologs of TAC1 gene in other species. TAC1 gene was observed in 26 species of vertebrates. Multiple sequence alignment was then performed for subsequent computational analysis. To find duplicates or paralogs of TAC1 in human genome, UCSC (University of California Santa Cruz) genome browser (www.genome.ucsc.edu) was used. It uses BLAT (BLAST like Alignment tool) for the sequence alignment of proteins and DNA. A gene is considered duplicate if it has an additional hit on the genome and as singleton if it has only one hit on the genome. MAFFT, online version 6.0 (http: // align.bmr.kyushu-u.ac.jp/mafft/online/server/), was employed for multiple protein alignments using the E-INS-i strategy with default parameters. To estimate the phylogenetic relationships of sequences distance-based analyses was performed using the Neighbor Joining (NJ) (a bottom up clustering method used for the construction of phylogenetic trees) programs. Predictions for TAC1 interactors were performed using the STRING 8.3 database (string-db.org), a database of known and predicted protein-protein interactions. The interactions include direct (physical) and indirect (functional) associations. They are derived from the sources of genomic context, high throughput experiments, conserved coexpression and PUBMED. Sequence logos were generated using a web-based program, Weblogo, version 3.0 (http: //weblogo.threeplusone.com/) developed by Crooks[23] and Schneider and Stevens[24]. A logo was generated with amino acid sequences from preprotachykinin 1 gene of 26 vertebrate species (Figure 7) including homo sapiens. It is a web based application designed to generate sequence logos. Sequence logos are a graphical representation of an amino acid or nucleic acid multiple sequence alignment. Each logo consists of stacks of symbols, one stack for each position in the sequence. The overall height of the stack indicates the sequence conservation at that position while the height of the symbols within the stack indicates the relative frequency of each amino or nucleic acid at that position.

3. Results

System level properties, such as gene duplicability, gene appearance in evolution, protein-protein interaction network and amino acid sequence conservation in prepro-tachykinin 1 precursor gene (TAC1) from 26 vertebrate species and the structure of its receptor NK-1R was studied (Figure 3). No additional hit for TAC1 gene on human genome was found by BLAT (Figure 4) analysis, suggesting that it is a singleton gene and a single copy in the genome consequently making it functionally essential. A small perturbation to this gene can lead to a fatal outcome. Protein-protein interaction network suggests that TAC1 gene doesn't interact with many proteins. It has only a few interactors which belong to the tachykinin family (Figure 5).

Human BLAT Results

BLAT Search Results

ACTIONS	QUERY	SCORE	START	END	QSIZE	IDENTITY	CHRO	STRAND	START	END	SPAN
browser details	Homo	358	1	129	129	99.8%	7	++	97361925	97369229	7305

Figure 4. BLAT results of human TAC1 gene

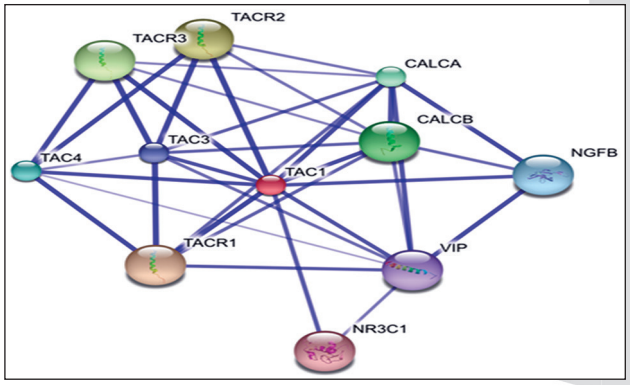

Figure 5. Protein-protein interaction of TAC1 gene by using STRING 8.3

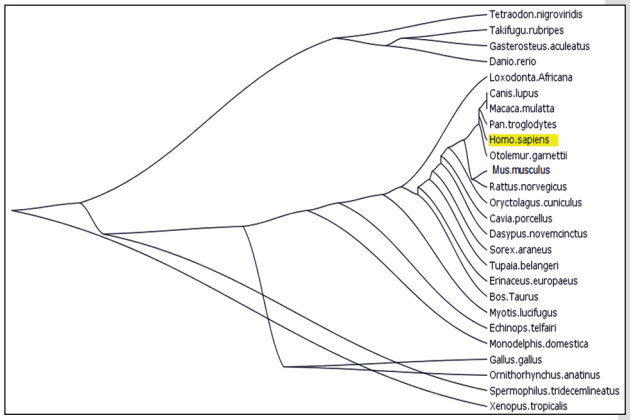

Figure 6. Phylogenetic tree of TAC1 gene by using MAFFT online version 6.0

The evolutionary tree (Figure 6) of TAC1 suggests that it appeared at the level of vertebrates and it is a new gene. It also suggests that TAC1 has a role specific to organisms with advanced nervous systems and that it is brain specific, required for sophisticated functions. We already know that TKs are present in both lower and higher vertebrates but they are much more advanced and authentic in mammals. They are also highly diversified and established in mammals where they are involved in regulation of several important functions such as developmental processes, differentiation of the cell, brain development, axon guidance, neurotransmission, immune modulation, mood control, behavioural responses, gastrointestinal contractions, lung and airway functions, response to environmental stress, learning and memory.

4. Discussion

There are two distinct conformational isoforms of NK-1R: a full-length NK-1R (NK-1RF) isoform and a truncated NK-1R (NK-1RT) isoform, which lacks the terminal cytoplasmic 96-aa residues [25]. Both of these isoforms have the same binding affinity for SP but different affinities for NKA. The NK-1R has a relatively long 5′ untranslated region compared to the other tachykinin receptors, which is preceded by a single TATAAA sequence[26]. TACR1 is the gene encoding for NK1R (Table 1; Figure 2)[27]. This gene is evolutionary conserved and has the same structure with five exons interrupted by introns in identical positions[28, 29] (Figure 2). TM1-3 is encoded by exon 1, TM4 by exon 2, TM5 by exon 3 and TM6, 7 by exon 4. This feature of G-protein coupled receptors to retain introns is very uncommon in this superfamily [30].NK-1R is expressed in both the central (CNS) and peripheral nervous system (PNS).

Sequence conservation is displayed as a sequence logo. Sequence logos were generated as described [31] by using WebLogo version 3.0 (http: // weblogo.threeplusone.com). In this representation, the relative frequency with which an amino acid appears at a given position is reflected by the height of its one letter amino acid code in the logo, with a total height at a given position proportional to the level of sequence conservation (see material and method). Tachykinin 1 (TAC1) encodes

Figure 7. Amino acid sequence conservation in the vertebrate prepro- TAC1 gene from 26 species

for SP, NP gamma, NPK, NKA and this preprotachykinin 1 sequence also shows signal peptide, C terminal flanking sequence and propeptide. Preprotachykinin is cleaved into mature peptides after differential splicing. The sequence in the purple box shows the highly conserved signature motif of the tachykinin family (Figure 7).

Evolutionary analysis suggests that TAC1 is a new gene and is present in advanced animals such as vertebrates specially mammals who have a well established nervous system (Figure 6). It also shows that these genes regulate important nervous system functions in these animals but not in organisms with a poorly developed nervous system. CGRP, Glutamate and VIP, which are found to be co-localized and co-released with TAC1 encoded product SP in trigeminal ganglion confirmed by previous studies [32], is also evident from protein-protein interaction analysis in this study (Figure 5).

Cancer genes have two properties: they have large networks and many proteins are interacting in their pathways and they are mostly singletons[33]. Although TAC1 gene is found to have altered expression in many cancers, it has a small network. Protein-protein interaction also suggests that the TAC1 gene mostly interacts with members of its own family. Its pathway is very small which suggests that either it doesn't has many interactors or that there are other interactors in its pathway not yet discovered and that further studies are needed to answer this question. We cannot confirm that TAC1 gene is a cancerous gene but we assume that it may be, and that other interactors may be discovered later.

Conclusions

As TAC1 is a singleton, it is a fragile point in the genome which means that if there is a mutation in this gene, there are no other genes with similar functions that can compensate for its function. This can lead to deleterious effects such as neuropathological disorders, mental disorders and gastrointestinal disorders since it is mostly localized in the brain and gut. If the genes and nuclei regulating nervous system development and cardiorespiratory control are affected, it may cause embryonic lethality and Sudden Infant Death Syndrome (SIDS) too as evident from our previous study [34]because TAC1 and the interacting genes are mainly expressed in the central nervous system.

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Results of high tibial open wedge osteotomy in primary varus and double varus patients

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Abstract

Introduction: Knee ligament injuries (ACL, PCL, PLC) can Couse lower limb varus deformity. This condition can be either primary varus or double varus malalignment. In the past Ligamentous injuries was one of the contraindications for tibial osteotomy. The aim of this retrospective study was to evaluate the results of open wedge high tibial osteotomy with medium-term follow-up in primary varus and Double varus patients.

Materials and Methods: A retrospective study was based on 29 patients who underwent open wedge high tibial osteotomy between Jan 2003 and Sept 2010. Inclusion criteria were active patients with age less than 60 years with varus deformity. patients with obesity (BMI>35), smoking, patellofemoral pain, lateral compartment injury, deformity greater than 20 degrees, less than 90 degrees of knee motion were excluded. In all patients, clinical (IKDC, Modified Larson) and radiological criteria were measured before and after surgery.

Results: In a multivariate analysis adjusting for preoperative status of each outcome Only significant differences were observed in Post operation DJD and tibial slope variables between the two groups.. IKDC and Moified Larson indices in double varus, primary varus had significant difference in before and after surgery. Although no significant difference between the two groups in postoperative indices, however, the double varus patients improved more than primary varus in their indices.

Conclusion: It seems that the results are similar in each group and open wedge high tibial osteotomy can be effective in the treatment of double varus patients as well as primary varus. In double varus patients high tibial open wedge osteotomy can reduce their need to ligament reconstruction.

Key words: medial compartment knee arthritis, open wedge high tibial ostetomy, primary varus, double varus

Introduction

Knee ligament injuries (ACL,PCL,PLC)can cause lower limb malalignment (1) These changes are divided into three groups: primary varus malignment following medial meniscus and articular cartilage injuries which cause varus malalignment in tibiofemoral joint. Double varus malignment in which In addition, to varus changes in tibiofemoral joint there is separation in lateral compartment caused by soft tissue injury. Triple varus which is caused by posterlateral side injury leads to varus and recurvatum deformity. This is the combination of double varus and posterolateral complex injuries which cause changes along the lower limb alignment posterolateral instability and increased in external rotation and hyperextension(2) A high percentage of patients with varus knee and ligament injuries may see adduction moment in their walking. Primary varus changes can be due to this adduction moment (4,3) In such cases ligament reconstruction without alignment consideration can lead to increased pressure on grafts(5,2). Ligament reconstruction without limb malalignmet correction will reduce the success rate of surgery and will couse recurrent ligament instability. Correction Osteotomy reduce stress on the medial compartment of the knee and improves the ligament reconstruction outcomes (8, 6) In many double varus cases high tibial osteotomy improves functional scores and reduce the need for ligament reconstruction (5, 2) The aim of this retrospective study was to evaluate the results of high tibial open wedge osteotomy with medium-term follow-up in primary varus and Double varus patients.

Materials and Methods

Patient's selection

This is a retrospective cross section study which was based on 29 patients who underwent open wedge high tibial osteotomy between Jan 2003 and Sept 2010. Inclusion criteria were high physical activite age less than 60 years with varus deformity. Patients with BMI>35, smoking, patellofemoral pain, lateral compartment injury, deformity greater than 20 degrees, less than 90 degrees of knee motion were excluded from this study.

Preoperative evaluation

Amount of pattelofemoral and tibiofemoral arthritis, as well as lateral compartment integrity was assessed by antero posterior, lateral and axial weight bearing knee radiographs. Grade of DJD before and after surgery were evaluated based on Kellegren and Lawrence method(9) In double varus patients stress view were taken and compared with normal side to assess ligament injuries in the lateral compartment. Alignment view X-ray was taken in all patients before and after surgery. Mechanical angle obtained by drawing a line from the center of the femoral head to the center of the knee and ankle center therefor. amount of varus deformity was assessed by these lines(10) In double varus patients MRI was used to study ligament injuries of the knee. (11) tibial slope was measured before and after surgery by Noyes method in which this angle is measured between medial tibial plateau and proximal tibial anatomic axis (12) open wedge high tibia osteotomy in patients was done to transfer the weight-bearing axis of lower limb to 62% of the tibia plateu width in mild degree of arthritis and 50% of the tibia plateau width in symptomatic patient without arthritis (13) mechanical axis was corrected to 3 to 6 degrees valgus according to Dugdale method. The amount of pain decrease and patients functional scores was determined by IKDC and Modified Larson indices before and after surgery in both groups.

Surgical technique

Surgery was done under general anesthesia and in supine position.in patients with chondral lesion, meniscal pathology and loose bodies before surgery knee arthroscopy was done. After longitudinal incision in proximal tibia superior border of pes anserinus was identified and retracted then superficial MCL was completely released. two guide pin proximal to tibial tuberosity was inserted from medial side to lateral side to proximal tibiofibular joint under C-arm control. Then osteotomy was done and was opened according to pre op planning. Gap was filled by autologous bone graft and T.buttress plate was applied in all patients.(12)knee range of motion was begin immediately after surgery after 6 week they had partial weight bearing and after 10 week full weight bearing was applied.

Results

Clinical results

29 cases (35 knees)with varus deformity were studied. mean age was 27.63 (SD, 10.88) 18 patients (21 knees) have primary varus (6 male, 12 female). 11 patients (14 knees) have double varus with ligament instability (8 male, 3 female). 10 patients (27.7%) had ACL, PCL, PLC injuries.in all patients high tibial open wedge osteotomy was done.no patient had ligament reconstruction.. In patients who had involvement of both knees, both knees were operated with an interval of 6 months. Follow up time was between 2 to 7 years (mean 4.33year). IKDC index improvement in primary varus was 5.49 points, and in double varus patients was 7.9 points. No significant differences were observed in both groups before and after surgery in this index. Modified Larson index improvement in primary varus patients were 4 points and in double varus patients were 5.9 points (table 1)

Radiologic results

In primary varus patients Mean correction of anatomic axis of tibia and femur was 13.9° and in double varus patients was 14°. In primary varus patients mean correction of mechanical axes of tibia and femur was 15.47 ° and in double varus patients was 12.71°. Mean tibial slope changes in primary varus patients were 1.1° and in double varus patients were 2.7°. Posterior tibial slope was increased overall in this study. (Table 1) Comparison between the two groups in the variables significantly different was seen in Post operation DJD grade. In a multivariate analysis adjusting for preoperative status of each outcome Only significant

differences were observed in Post operation DJD and tibial slope variables between the two groups. in 90.5% double varus patients had no increase in DJD grade during follow up and 50% of double varus patients had no increase in DJD grade. Long term longevity score of high tibial open wedge osteotomy in primary varus patients for 6 year was 100%and in double varus patients this score for 6 year was 57% (Figure 1). There were no difference between insall-salvati and black -burn index before and after operation in both groups.

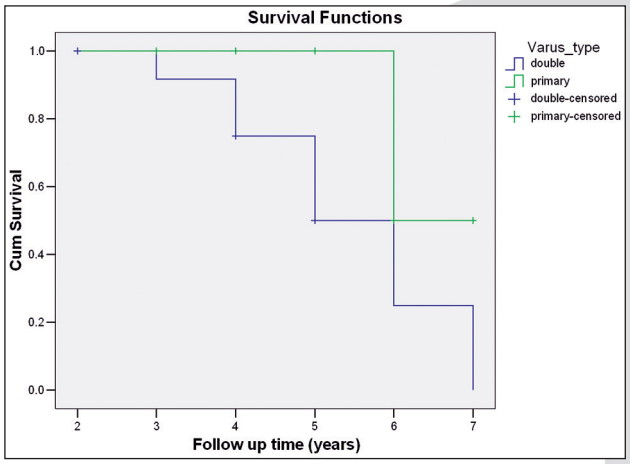

Figure 1. Survival curve for remaining in stable status

Discussion

Open wedge high tibial osteotomy is a known treatment in medial knee compartment osteoarthritis in young and active individuals with knee varus deformity. Although in the past ligament instability was one of surgical contraindications but today osteotomy has its own special place. This study findings shows that in spite of previous believe open wedge high tibial osteotomy in double varus patients with ligament instability will improve mechanical and anatomical axes and functional scores of the patients. There is no statistically significant difference in functional outcome of these patients as well as primary varus patient (IKDC ,Modified larson index). this shows that open wedge high tibial osteotomy will increase functional score and clinical symptoms of double varus patients as well as primary varus patients. Although there was no significant difference in post operation IKDC and Modified Larson indices between two groups but this indices had more improvement in double vaus patients. This reflects the fact that open wedge high tibal osteotomy in Double varus patients further improve the clinical symptoms.

Table 1. The results of open wedge high tibial osteotomy in primary varus and double varus patients

Variable	Varus type			
	Single		Double	
	Mean	SD	Mean	SD
Pre operation DJD grade	.76	(.94)	1.21	(.70)
Last DJD grade	.86	(.91)	1.79	(.70)
pre AFT Degree	8.48	(5.63)	6.86	(3.32)
post AFT Degree	-5.48	(3.72)	-6.69	(3.40)
Pre MFMT degree	12.00	(7.14)	9.43	(4.67)
post MFMT degree	-3.37	(4.60)	-3.29	(3.56)
tibial slope pre operation	10.00	(4.64)	10.86	(3.25)
tibial slope post operation	11.10	(4.58)	13.57	(2.34)
Location WBL pre operation	1.29	(.46)	1.57	(.65)
Location WBL post operation	2.48	(.60)	2.64	(.63)
Index insall preoperation	1.12	(.16)	1.02	(.14)
Index insall post operation	1.07	(.13)	1.07	(.18)
Black burn preoperation	1.12	(.28)	.97	(.36)
Black burn post operation	1.13	(.23)	.96	(.35)
Score IKDC preoperation	85.50	(16.39)	82.77	(12.57)
score IKDC post operation	90.99	(15.22)	89.86	(11.99)
Index larson pre operation	88.30	(11.21)	84.92	(12.78)
Index larson post operation	92.30	(9.92)	90.83	(9.29)

Lattermann et all for the first time studied 30 young active patients with ligament instability during 10 years follow up. They did osteotomy in one group, osteotomy with ligament reconstruction in another group and Osteotomy and ligament reconstruction with 6-month interval in the third group. According to IKDC score pain was major problem in all patients and no patient was symptom free. based on their study in patients ≥ 40 years osteotomy alone (improved IKDC and Modified Larson) had good clinical results and if the patients had instability in follow up ligament reconstruction was done 6 to 12 month later.(13)there was also good clinical results(IKDC ,Modified Larson index) in osteotomy alone in double varus patients in our study and results was the same as in primary varus patients. Although there was no significant difference between post operation IKDC and Modified Larson indices between two groups but double varus patients had more functional improvement than primary varus patients.

Noyes et all, studied 41 active young patients with varus deformity and ACL ,PLC injuries who underwent open wedge high tibial osteotomy with ligament reconstruction during their follow up (4.5 years)Pain was reduced in 71% of patients. 85% of patients had no giving way 66% had resumption of previous activities. 37% of patients had excellent knee score 34 % had good score. Cincinnati knee score significantly increased from 63 to 82. and mechanical axes correction was done in 80% patients. They recommended adding osteotomy to ligament reconstruction(2)

Badhe et all, studied 14 varus patients with ligament instability who underwent open wedge high tibial osteotomy.5 patients with double varus had closed wedge high tibial osteotomy and ligamet reconstruction. 9 patients had triple varus deformity in which 5 patients under went open wedge high tibial osteotomy with ligament reconstruction and 3 patients had osteotomy only ,in mean follow up of 2.8 year 86% of patients were stable one patient had severe infection 93% of patients could participate in their physical activity but none of the patients were able to return to competitive sports.35 %of patients had some degree of pain and Cincinnati score was improved from 53 to 74 in their study 2 patients had poor results 4 patients had acceptable results and 8 patients had good results. in triple varus patients open wedge high tibial osteotomy had better results than closed wedge osteotomy.(5) long-term survival of open wedge high tibial osteotomy were between 7 to 10 in different studies. Agility et all have mentioned that open wedge high tibial osteotomy survival for 7 years is 88%(14)and Ritter MA et all showed that osteotomy survival for 7 year is 58%(15).these studies mentioned that open wedge high tibial osteotomy will post pone the need for joint reconstruction at least for 7 to 10 year.in this study long term survival of open wedge high tibial osteotomy in primary varus patients for 6 year was 100% and in double varus patients 6 year survival rate was 58%(figure 1) which was the same as other studies. Overall, 10% of those with primary varus and 50% of double varus patients had increase in DJD grade in their follow up. Jason et all , in their study found that patellar tendon length (pre op and post op insall index)doesn't change in follow up. This reflects that patellar position changes is because of structural cause's and biological factors such as tendon scars has no effect on tendon length(16) In this study, the patellar tendon indices (insall-salvati,black –burn)did not significantly change after surgery and this confirms that issue (table 1)

Limitation: we had difficulty in taking more sample size and following the patients, so we suggest to conduct such study using a multicenter design and with a unified protocol. Establishment of patient registry can have a significant role in following of such patients.

Conclusion

Over all this study confirms that open wedge high tibial osteotomy results are similar in each group and can be effective in the treatment of double varus patients as well as primary varus patients. In double varus patients high tibial open wedge osteotomy can improve functional scores and will reduce their need to ligament reconstruction.

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Author's contribution

Mahmoud Jabalameli; contributed to performance of procedures and development of protocol

Mohammad Rahbar and Mehran Radi; contributed to the development of protocol and abstracted data and prepared the manuscript

Hosseinali Hadi; made substantial contribution in study conception and abstracted data

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Quantitative evaluation of the mast cell population in border of ulcers in America Cutaneous Leishmaniasis – ACL

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Abstract

American Cutaneous Leishmaniasis (ACL) is an infectious disease with a broad spectrum of presentation, has two clinical poles, one anergic and other hyperergic. The inflammatory response to the etiologic agent is complex and involves several cell lines, especially lymphocytes, plasma cells, histiocytes, and antigen-presenting cells. Other cells are observed in inflammatory exudate in a lesser degree, such as eosinophil and mast cells – MC. Several studies have sought to investigate the role of mast cells in cutaneous Leishmaniasis. This study evaluated 64 skin biopsies of leishmanial ulcer edge. The number of mast cells and leishmania were observed in the timeline. It was found that the number of mast cells and leishmania decline with age of the lesion, there is a negative correlation $p<0.05$. In the late stages of injury (more than eight weeks) the number of mast cell sin the leishmanial lesion sis almost nil. The authors question whether there is really involvement of the mast cells in the chronic phase of Cutaneous Leishmaniasis.

Key words: Mast Cell, Leishmaniasis, Cutaneous Leishmaniasis.

1. Introduction

American Cutaneous Leishmaniasis (ACL) is a group of infectious diseases with the clinical spectrum of presentation similar to that seen in leprosy, ranging from one pole hyperergic to another anergic (24). The inflammatory response to the etiologic agents complex and involves several cell lines, especially lymphocytes, plasma cells, histiocytes, and antigen-presenting cells (25, 26). Other cells are observed in inflammatory exudate in a lesser degree, such as eosinophil and mast cells - (09, 27 and 28). Knowledge about the histophysiology of mast cells and eosinophil in recent years, has gained attention from researchers. The role of these cells in the body homeostasis, inflammatory response and infections has been described (28,29, 30, 31 and 32). Studies has shown a complex machinery, present especially in mast cell involved in the production of complex molecules that regulate the vascular diameter, leukocyte migration, as well as intervening in the modulation of immune response in the skin and mucous membranes(33,34,35). This knowledge has allowed mast cells to be added as part of inflammatory response, including the inflammatory phenomena determined by biological agents. This study evaluates the mast cells population in the early and late phase of the ACL, discussing the results based on the current literature, speculating what would be the role of this cell in the afore said morbidity.

2. Materials And Methods

Ethical approval for this study was obtained from the relevant local ethics committees (Ethics committee of the Medical School of the Federal University of Ceará in Barbalha).

Tissue specimens and patients

Data relating to patients were obtained from Outpatient Tropical Medicine at the Federal University of Ceará, Campus Cariri, Ceará, Brazil. The health records of patients were retrieved and sociodemographic and clinical data were analyzed (n=64, male: female ratio =1.56; group mean age =37.46875 ±19.84381). The diagnoses of patients presenting ACL were confirmed by clinical examination, Montenegro intradermoreaction (IDRM), culture with NNN media and histopathological analysis in the Laboratory of Experimental Pathology (LAPEX) from College of Medicine at the Federal University of Ceará, Campus Cariri,

Ceará, Brazil. The archived tissue blocks from surgically resected samples (n=64) at LAPEX were evaluated in this study.

Histological, histochemical staining and Immunohistochemistry for identification of mast cells and Leishmania

For the purposes of morphological and histochemical analysis, samples were fixed in formalin, embedded in paraffin, serially sectioned at 05 microns and evaluated under a conventional light microscope. Sections were stained with hematoxylin and eosin (H&E) for routine examine. In order to visualize mast cells, two sections of each sample were stained with 1% toluidine blue and counter stained with 5% methanol yellow for 5 min, following which the sections were dehydrated, cleared and mounted with synthetic balsam. The sections were stained with Giemsa for Leishmania visualization and count (01).

For the Immunohistochemical evaluation of Mast cell,in order to compare the histochemical count, sections were mounted on organosilane-coated slides (Dako Silanized Slides Code No. S3003). The primary mouse monoclonal antibody against Tryptase Antigen (Clone AA1 - Isotype IgG1, kappa, to formalin use of the DAKO CORPORATION) was detected with the aid of an ELITE ABC KIT (product # K0690, Vector Laboratories, Inc. 30- Ingold Road, Burlingame, CA 94010 – USA), employing chromogen diaminobenzidine for colour development. Slides were then counter stained with Mayer's hematoxylin and mounted. Negative controls were obtained by substituting normal whole rabbit serum (product # X0902; Dako) for the primary antibodies. A sample of the normal skin (shown previously to be strongly positive to MAST CELL) was used as a positive control.

For the Immunohistochemical evaluation of Leishmania, in order to compare the count of histochemical (Giemsa); four-micron thick histological sections were collected on previously prepared slides (Dako Silanized Slides Code No. S3003); then deparaffinated in xylene and rehydrated in ethanol and water were performed. The endogenous peroxidase activity was blocked. Antigenic retrieval was done in a 96°C water bath withcitrate buffer (Dako target retrieval solution), inhibition of the specific bonds with skimmed milk (30mlTRIS + NaCl + 0.3g of bovine albumin Merck + 0.3gMolico skimmed powdered milk). After draining the milk, the primary antibodies Anti-Leishmania polyclonal antibody (diluted at 1/4000) in diluting solution with a Dako unspecific bond reducer were used, then placed in a humid chamber at room temperature for 30 minutes. The biotinylated secondary antibody from an LSAB kit DAKO was used. The development (antigen-antibody bond) was visualized withdiaminobenzidine, Dako DAB+ kit, the slides counter stained with Harris' hematoxylin, and mounted. Anti-*Leishmania* serum was produced at the Leishmaniasis Surveillance Laboratory of Evandro Chagas Clinical Research Institute (IPEC), FIOCRUZ, by immunization of albino rabbits inoculated with four doses of soluble protein extracts of promastigotes forms of *Leishmania (Leishmania) chagasi* (MHOM/BR/1974/PP75).

Determination of mast cells

Mast cells was identified on the basis of cytoplasm with intense purple granules and nuclei presenting a bluish morphological aspect with Toluidine method (figure 01), and cytoplasm with brown colour at Immunohistochemistry (figure 2). For quantitative analysis, an Optical microscope Olympus -BH2 (model CX31, RTSF, Miami, FL, USA), with · 10 ocular and · 40 objective lenses, was employed, and an ocular lattice (area 0.092 mm2) with 100 points composed of 10 horizontal and 10 vertical test lines was superimposed on the test field to be measured. A total area of 1.84 mm2 was evaluated for each of the samples, and this corresponded to 20 randomly selected high-power microscopic fields located in regions of high mast cells densities.

Determination of Leishmania

The Leishmania stained with Giemsa were identified as small body vacuolated with blue kinetoplast, observed within macrophages or scattered in tissue (figure 4). In the immunohistochemistry small brown point were observed (figure 03). For quantitative analysis, an Optical microscope Olympus - BH2 (model CX31, RTSF, Miami, FL, USA), with · 10 ocular and · 40 objective lenses, was employed, and an ocular lattice (area 0.092 mm2) with 100 points composed of 10 horizontal and 10 vertical test lines was superimposed on the

test field to be measured. A total area of 1.84 mm2 was evaluated for each of the samples, and this corresponded to 20 randomly selected high-power microscopic fields.

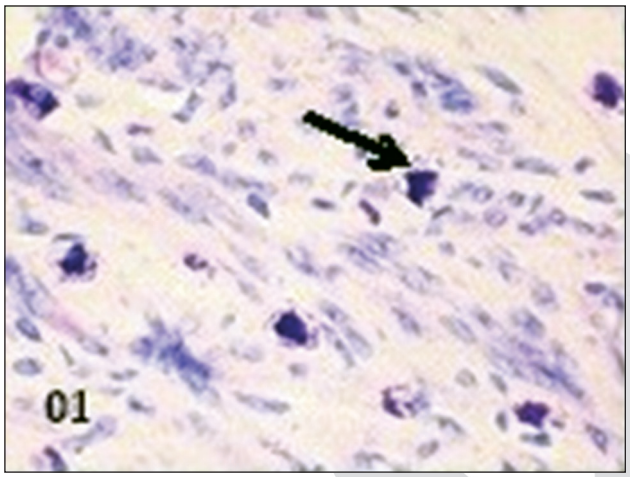

Figure 1. 01. Mast cell stained by toluidine blue

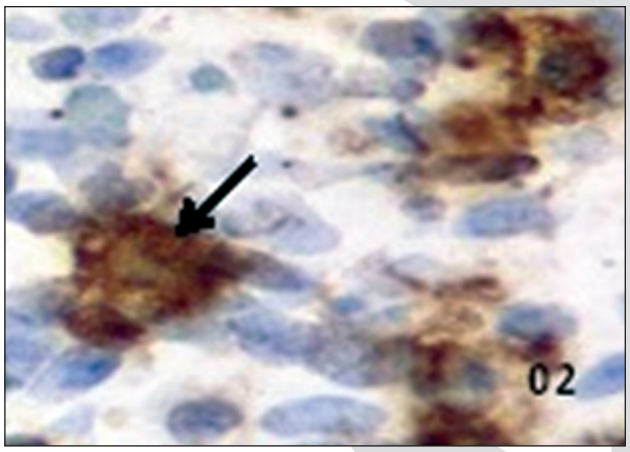

Figure 2. 02. Mast cell by immunohistochemistry

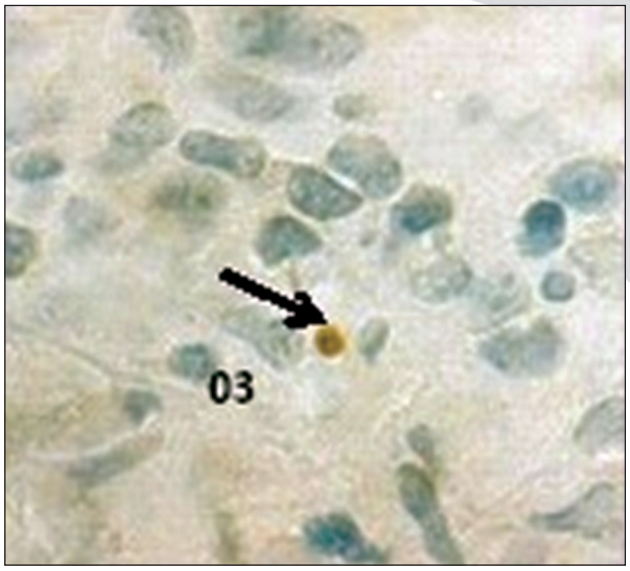

Figure 3. 03. Leishmania by immunohistochemistry

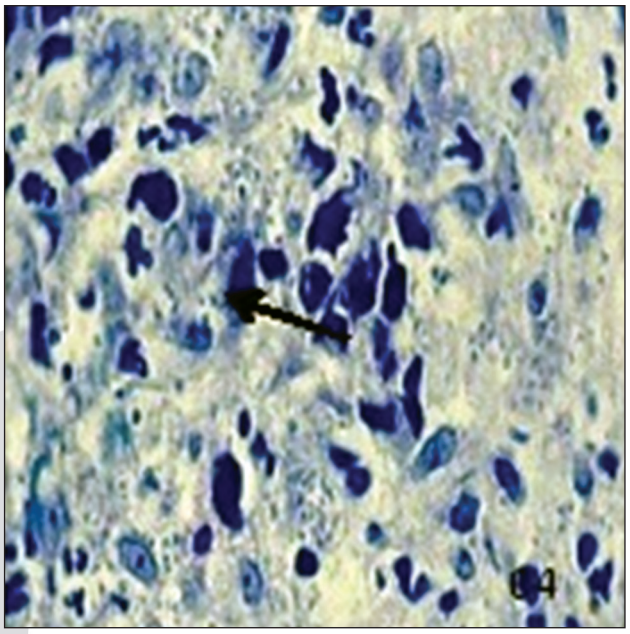

Figure 4. 04. Leishmania stained by Giemsa

Statistical analysis

Data were tabulated in Microsoft Excel spread sheet (2010). A data base was performed with theEpi InfoVersion 3.5.3 releaseby CDC in January 26, 2011 and GraphPad Prism, version 3.00, mar 25, 1999. The comparison between counts made by histochemistry and immunohistochemistry were performed using paired sample t-test, considering ap<0.05. The comparative analysis between the results obtained forthe count of mast cells and Leishmania was performed using the Pearson's coefficient considering a p< 0,05.

3. Results

The study population was predominantly male and living in rural areas as shown in Table 1. The youngest patient was 09 years old, the oldest 85. The average age of cases is around 37.4 years. The study population is made up of brown (n=43, 67.20%), blacks, (n=04, 6.20%) and whites (n=17, 26.60%). Farmers (n = 37) account for half the population (50%). The progression of the lesions was divided into recent and late. Recent injuries are considered the lesions with down to 08 week sat diagnosis, late lesions with more than 08 week sat diagnosis. The distribution of the time evolution can be observed in Table 2.

The number of the Mast Cells in the lesions decreased over the weeks, as show in figure 05. This

Table 1. Epidemiological variables of the population

		N(64)		N(100%)	
Male	Female	42	22	66	34
Rural	Urban	60	04	94	06

Table 2. Evolution in weeks at diagnosis

Weeks	N(64)	N(100%H)	MAST1*	LEISH1*	MAST2**	LEISH2**
02	04	06,25%	255	192	253	133
03	05	07,90%	276	169	275	129
04	10	15,42%	439	272	438	190
05	05	07,90%	172	96	172	57
06	04	06,25%	113	58	112	38
08	06	09,40%	142	74	142	48
Recent	34	53,12%	1397	861	1392	595
09	08	12,42%	150	66	148	29
12	06	09,40%	91	27	90	09
16	05	07,90%	59	14	59	04
20	03	04,68%	31	03	31	00
22	03	04,68%	20	03	20	00
25	02	03,12%	04	00	04	00
28	01	01,56%	01	00	01	00
30	01	01,56%	00	00	00	00
32	01	01,56%	00	00	00	00
Late	30	46,88%	356	113	353	42

**Histochemical staining. ** Immunohistochemistry.*

decreased observed has a negative linear correlation (Pearson's Coefficient = -0,85061, 95% confidence interval: -0.9068 to -0.7647 with P<0.0001; Strong Negative Correlation), (36).A similar aspect was seen with the number of the Leishmania. The Leishmania reduce in number over time, figure 05.This reduction has a negative correlation (Pearson's Coefficient = -0,78745, 95% confidence interval: -0.8657 to -0.6716 with P<0.0001; Strong Negative Correlation), (36).

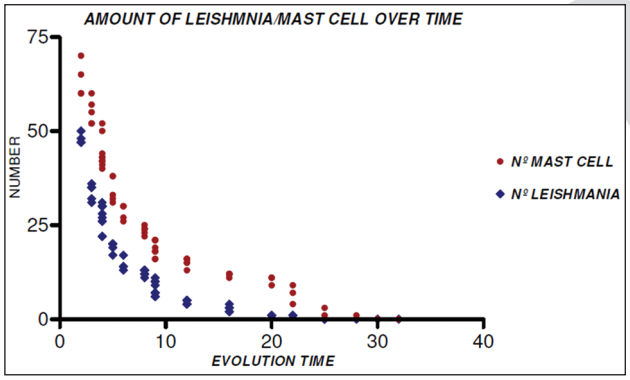

Figure 5. Amount of Leishmania/Mast cell over time

The data collected show that the number of Mast Cells and Leishmania decrease with time of infection and there's a strong correlation between the reduction of Leishmania and the number of mast cell (Pearson's Coefficient = 0,9831, 95% confidence interval: 0.9068 to -0.7647 with P<0.0001).

There was no statistical difference between the count of mast cells in histochemistry and immunohistochemistry. On the other hand count for Leishmania the immunohistochemistry was more sensitive than histochemistry.

4. Discussion

The experimental model of the American Cutaneous Leishmaniasis caused by *Leishmania Mexican*, determines a progressive disease which rapidly spreads in the susceptible BALB/c mouse, whereas in C57BL/6 mice the disease tends to be self-healing. The first model it has been established that Th1 cytokines lead to disease resistance. On the other hand in the BALB/c mice early presence of IL-4 and IL-10 determine a Th2 response and disease

progression (04).The cells responsible of producing these IL-4 and IL-10 cytokines in the early phases of the disease remain controversial, although cells of the innate immune system are a likely source. The mast cells are possible candidates that produce these cytokines (06, 07). Besides, due to their location in the skin and mucosa, mast cells are one of the first cells to encounter invading pathogens. They are found in association with blood vessels, as well as in tissues and surface exposed to external environment such as skin and mucosal linings, which are common portals of infection (11). This fact would have an important role in the early stages of Leishmaniasis, say some authors (07, 08). In experimental model of Visceral Leishmaniasis an interleukin-3-dependent augmentation in mast cell committed progenitors is observed in BALB/c but not in C57BL/6 mice during *Leishmania* infection. The mast cell supernatants inhibit IFNg -dependent restriction of *Leishmania* growth in macrophages in BALB/c mice whereas the reverse phenomenon occurs in C57BL/6 mice. Most of this data point to a possible involvement of mast cells in cutaneous infection by Leishmania, although KATAKURA, 1993, demonstrated that the evolution of the Cutaneous Leishmaniasis is independent of the Mast cell (09).

In this work we observed the presence of mast cells in regular issue in the early stages of skin lesions. The number of mast cells decreased over time. The old lesions showed a smaller number of mast cells. Reducing the number of mast cells over time showed a direct correlation with the decrease in the number of Leishmania in the wound.

The reduction of mast cells over time and maintenance of the disease creates a question: It would really bethe mast cells one the initiators and maintainers of the lesion in Cutaneous Leishmaniasis? SAHA,2004, demonstrates that *Leishmania donovani* is responsible for the modulation of the mast cells which would explain the reduction of these cells in the timeline (10). In the same way *Leishmnaia (Viannia) braziliensis "invitro"* may activate the Mast Cell and also modulate the cytokines production, especially IL-4 (12).In present study none of the cases of the recent lesion (injury less than eight weeks) was observed absence or reduced number of mast cell. The normal number of mast cells in the skin was used the data provided by JANSSENS and associates, 2005 (13). On the other hand the number of mast cells gradually reduces with age of the lesion. This observation suggests that even though the mast cell is a cell involved n the early stages of ACL, probably should not be in the late phase of the skin lesion. Who would be responsible for the maintenance of the lesion where as mast cells and Leishmania reducer significantly in the late phase of Cutaneous Leishmaniasis?

Sakaguchi and associates (16,17) reactivated interest in the concept of T-cell-mediated suppression around the year 1990 by showing that a minor population (<10%) of CD4+ T cells, which co-expresses the interleukin-2 receptor (IL-2R) α-chain (CD25), is primordial for the control of auto reactive T cells *in vivo.* Subsequent *in vitro* studies by several groups showed that CD4+CD25+ T cells are both hyporesponsive and suppressive (18, 19). CD4+CD25+ T cells were discovered originally in mice, but a population with identical phenotypic and functional properties has been defined recently in humans (20, 21).Studies have related the presence of this cell as the main stay in the maintenance of some diseases, especially Leishmaniasis. Considering that it produces IL4 and especially IL10. Thus the presence of mast cells in the maintenance of the lesion would not be essential in the late phase of the Cutaneous Leishmaniasis, corroborating the findings of this study (15, 22, and 23).

In the present study also found that the use of Giemsa stain to identify Leishmaniain late lesions (more than eight weeks) is not a good method. The immunohistochemistry was more sensitive and statistically representative.

5. Conclusions

1. The number of mast cells in Leishmanial infection reduces numerically overtime;
2. The number of Leishmania in skin lesion reduces numerically with the passage of time;
3. There is a positive and statistically significant correlation with the simultaneous reduction of mast cells and Leishmania in skin lesions leishmaniotic, whose cause is unknown;
4. The search for Leishmaniain cutaneous lesions of Leishmaniasis with more than 8 weeks should be discouraged.

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Treatment of clinically significant diabetic macular edema (CSME): Laser therapy alone or laser with Anti-VEGF

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Abstract

Background and purpose: Clinically significant diabetic macular edema (CSME) is a severe form of non-proliferative disease reveals in the retinal photoreceptors dysfunction. We designed this clinical trial to compare two methods of treatment in CSME, laser therapy alone and laser with Anti-VEGF (Bevacizumab).

Methods: In this randomized clinical trial with a matched pair parallel design, 76 diabetic patients with CSME were included. Local ethical committee confirmed the study and informed consent was taken from all patients. Baseline visual acuity was measured with Snellen chart. One eye was treated by laser therapy and the other was treated with a combination of laser and anti-VEGF therapy (Bevacizumab; 0.05 mL). Visual acuity was measured six weeks later. Data were entered into the SPSS-19 software. Analysis of data was done using paired t-test and Mc-Nemar test.

Results: The combination therapy with laser and anti-VEGF was done in 58 (76.3%) of right eyes and 18 (23.7%) left. In fundoscopy, 57 (75%) had macular edema and 19 (25%) had both macular edema and macular ischemia. Visual acuity was reported to be significantly different between two groups after the treatments.

Conclusions: Anti-VEGF therapy combined with laser could be more effective in improving the macular edema in diabetics.

Key words: Anti-VEGF (Bevacizumab), Clinically significant diabetic macular edema (CSME), Laser therapy, Visual acuity.

Introduction

As industrialization of world is going faster and immobile lives are extending more than any other time, diseases related to sedentary life like diabetes mellitus is increasingly disturbing all populations (1).

Clinically significant diabetic macular edema (CSME) is a disease which result from swelling of extracellular layer in the macula, and intra- and extracellular edema from breakdown of the blood–retinal barriers and reveals in dysfunction of the retinal photoreceptors (1-3).

At the present time, there are two main methods of non-surgical treatment for this problem: macular laser photocoagulation and medical therapy. As the large randomized trials showed previously, laser therapy is the most cost-effective method for these kinds of patients. On the other hand, vascular endothelial growth factor (VEGF) has an important role in the pathogenesis of proliferative diabetic retinopathy and diabetic macular edema, so it has been proposed that inactivation of extracellular VEGF could be considered as potential treatments for diabetic macular edema (1, 4).

We designed this clinical trial to compare two methods of treatment in clinically significant diabetic macular edema (CSME), laser therapy alone and laser with Anti-VEGF.

Materials and methods

In this randomized clinical trial with a matched pair parallel design, all diabetic patients who referred to retinal clinic of an academic hospital in Gorgan, Northeast of Iran; diagnosed as having diabetic retinopathy and CSME were included into the study. Inclusion criteria were a diagnosis of bilateral CSME and a similar macular thickness (mean differences of 10 micron) in optical tomography (OCT). Local ethical committee confirmed the study and informed consent was taken from

all patients. History taking, examination with slit lamp, fundoscopy, measurement of the macula thickness with optical tomography (OCT) and confirmation of CSME were done consequently. Baseline visual acuity was measured with Snellen chart. The mean differences of macular edema in right and left eye were recorded. Demographic and baseline data such as sex, age, duration of diabetes and the type of diabetes were recorded, too.

Selection of eye was randomized by patients and which eye were treated by laser alone or combination therapy was depended on patient decision. One eye was treated by laser therapy and the other was treated with a combination of laser and anti-VEGF therapy (Avastin: Bevacizumab; 0.05 mL), according to the patient preference. The injection was done with a 30 gauge needle from the supratemporal area, 4 mm posterior to the limbus. Visual acuity was measured with Snellen chart six weeks after the treatment.

Data were coded and entered into the SPSS-19 software. Analysis of baseline variables like visual acuity and the outcome distribution in the follow-up time was done using paired t-test and Mac-Nemar test. P-value was considered significant if less than 0.05.

Results

Table 1. Fundoscopy results in patients referred with CSME

Macula Mean thickness ± 10 (µm)	Number	%
251-300	6	7.9
301-350	23	30.3
351-400	28	36.8
401-450	15	19.7
451-500	4	5.3
Total	76	100

In this clinical trial, 76 patients fulfilled the inclusion criteria with a mean (±SD) age of 57.2 (±11.9) years (min=32 and max=83). There were 37 females and 39 males (Male/ Female:0.95). Most of the patients were diabetes type 2 (N=71, 93.4%). Mean (±SD) of diabetes duration was 11.3 (±4) years (range:5-25). In most of the patients mean macular edema was in the range of 301-400 micrometer. Fundoscopy results are shown in table 1.

Due to the randomization method level in this study which was the two eyes of one person, the best equality of distribution in confounding variables was achieved in here. On the other hand, the distribution of all variables (demographic and confounding) was equal in both groups, so there is no need to compare these variables between two groups.

The combination therapy with laser and anti-VEGF was done in 58 (76.3%) of right eyes (OD) and 18 (23.7%) of left eyes. In the cup examination, 57 (75%) had macular edema and 19 (25%) had both macular edema and macular ischemia.

Visual acuity was reported to be significantly better in combination therapy in the follow-up after treatments (P-value< 0.05).

Discussion

Visual acuity after the surgery showed a significant statistical difference between the two groups and was better in the combination group (0.50 ±0.25) vs the laser group (0.36 ±0.19).

Haritoglou et al in a study treated their patients with 0.05 ml of 1.25 mg Bevacizumab. After 6 weeks follow ups, the mean visual acuity increased to 0.75 ±0.37. They concluded that in those with a wide spread diabetic macular edema who do not responding to other treatments, an improved visual acuity and reduced retinal thickness could be achieved after intra-vitreal injection of Bevacizumab (5), which was similar to our results.

Table 2. Visual acuity before and after treatment in the two groups treated with Anti-VEGF combined with laser or laser therapy alone

Variables	Laser and Anti-VEGF	Laser	P value
VA before treatment (in decimal)	0.03 ± 0.12	0.12 ± 0.03	1
VA before treatment (LogMAR)	0.08 ± 0.93	0.08 ± 0.93	1
VA after treatment (in decimal)	0.36 ±0.19	0.05±0.25	<0.001
VA after treatment (LogMAR)	0.51±0.25	0.37±0.25	<0.001

Gulkilik et al. designed a study in Turkey (2020) to assess the effect of intra-vitreal injection of Bevacizumab on the retinal neovascularization and diabetic macular edema (DME) resistant to laser photocoagulation therapy. The mean of best-corrected visual acuity (BCVA) was significantly better than baseline only at week 2, and the mean of central macular thickness (CMT) was significantly decreased at the first, second and fourth weeks compared to the baseline (6). This was similar to our results, too.

Kinge et al (2010) followed up their patients for 3 months and reported a significant difference between BCVA and CMT of the control group and those treated with Ranibizumab. Monthly Ranibizumab significantly reduced the macular edema and increased BCVA in patients with central Retinal vein occlusion (CRVO) (7). This was similar to our results.

Romano et al (Italy, 2010) reported that combination therapy could reveal to a persistent morphologic idiopathic polypoidal choroidal vasculopathy (IPCV) lesions, rapid reduction of macular thickness and returning of the size of vascular polipoid lesions (8).

Fard et al (2010) measured the prophylactic effects of intravitreal injection of Bevacizumab during cataract surgery on the thickness of retinal afterward in diabetic retinopathy. They reported a significant increase in central macular thickness in the control group after one month but no increase was seen in Bevacizumab. Six months later there had been seen no significant difference between two groups regards to the macular thickness and visual acuity. Authors suggested this treatment for a short course therapy after the operation (9).

Sun et al. (2010) suggested that the best treatment should be a combination of anti-VEGF and other drugs and laser to reduce the risk of several injections and have the optimum results (10).

The advantages of anti-VEGF therapy are discussed in other studies, too (11-15).

Conclusions

As we reported in this study, anti-VEGF therapy combined with laser could be more effective in improving the macular edema in diabetic patients.

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Determinants of child abuse and attitude of the families towards child abuse in the community in Pakistan

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Abstract

Background: Child abuse causes emotional scarring and long lasting effects throughout life, damaging a child's sense of self respect, ability to have healthy relationships with other humans, and ability to perform good at home, at school and at any profession.

Objectives: To find the determinants and risk factors of child abuse and attitude of the families towards child abuse.

Methodology: This cross-sectional study was carried out in 10 schools of Lahore and Center of Child abuse in Children Hospital Lahore for the period of 6 months. A total of 300 cases were included in the study having history of child abuse. Respondents of age<14 years of both gender and their family members were interviewed on Child Abuse Scale (CAS) after taking informed consent and demographic information was obtained on a pre-designed questionnaire. Data was entered and analyzed through SPSS 17. Mean ± SD was calculated for quantitative variables while frequency and percentages were calculated for qualitative variables. Groups were compared by using chi-square test for qualitative variables and t-test for quantitative variables. P-value of <0.05 was considered as significant.

Results: The mean ages of the respondents in years were noted as 11.95 ± 1.68 years. There were 136 (45.33%) male and 164 (54.67%) females. There were 270 student and 19 laborers. There were 50 (16.67%) abused sexually, 197 (65.67%) abused physically, 53 (17.6%) abused emotionally. There was significant difference between both genders (p-value = 0.000).

The study results showed that there were 119(39.67%) respondents injured after abuse. Respondents reported about 214(71.33%) men and 76(25.33) women abusers. There were 11(3.67%) respondents who had physical disability and 43(14.33%) had mental disability.

In 63(21%) respondent's mother had died and 83(27%) and in respondent's father had died. The disclosure of abuse to the family members caused anger in family members. There were 114(38%) respondents who were rejected and beaten by any family member on telling about child abuse; however 186(62%) respondents were believed and not beaten by any family member. Only 91(30.3%) respondents were brought to hospital for medical examination by any family member after child abuse. No legal action was taken by any family member against the abusers.

Conclusion: Thus it was concluded form this survey that females were at more risk of being abused, whether sexually or physically. Parents and teachers are required to give more attention to their children and students. Abused children can be cured by psychological therapies. The family attitude towards abused child is unsatisfactory and should be improved by health education.

Key words: child abuse, sexually, physically, emotionally, neglect.

Introduction

Child abuse is defined as the physical, sexual, emotional maltreatment, or neglect of a child.[1] Child abuse and neglect always leave permanent scars. Some of these scars are physical; however emotional scarring has long lasting effects. The trauma of child abuse resulted in poorperformance at home, at school and at any profession.

In the United States, the Centers for Disease Control and Prevention (CDC) and the Department of Children and Families (DCF) has defined child abuse as any action or series of actions by a parent or guardian or any other human that results in harm, risk of harm, or threat of harm to a child.[2]

Child abuse can occur anywhere for example child's home, organizations, schools, play grounds, markets or hotels. There are four major categories of child abuse: neglect, physical abuse, emotional abuse or psychological abuse, and child sexual abuse. According to a study, child abuse is "any recent act or failure to act on the part of a parent or caretaker which results in death, serious physical or emotional harm, sexual abuse or exploitation, an act or failure to act which presents an imminent risk of serious harm". [3]

There are strong associations between exposure to child abuse including physical or sexual abuse and higher prevalence of many chronic conditions in later life. The strongest evidence comes from the Adverse Childhood Experiences (ACE's) series of studies which show correlations between exposure to abuse or neglect and higher rates in adulthood of depression, anxiety, schizophrenia, hypertension, diabetes, drug addiction, road traffic accidents, high-risk health behaviors, poor quality of life and shortened life. The recent research studies had emphasized that exposure to child abuse represented a serious public health issue that should be addressed by the Government and citizens of a civilized nation.[4] Child abuse was described as a major life stressor that has consequences involving the mental health problems in children, adolescences and young adults. It has been identified that childhood sexual abuse is a risk factor for the development of distorted personalities with weak social relationships during adolescence and adulthood. [5, 6]

The early experiences of abuse during childhood can precipitate generalized anxiety disorder, bipolar disorder, mania and major depression. For example, adults with a history of child abuse, whether sexual abuse, physical abuse, or neglect, have more chances of developing depression then an adult who has never been abused.[7] Child abuse can also cause problems with the neurological development and personality development of a child. Abused children often develop deficits with speech problems, labile mood, irrational behavior and emotional disturbances.[8] These risks are elevated when child abuse is combined with trauma or fetal alcohol and smoking exposure. [5, 6]

When some of these children become parents, especially if they suffer from posttraumatic stress disorder (PTSD), dissociative symptoms, and other complications of child abuse, they faced difficulty with their infant and young children's needs and normative distress, which may in turn lead to adverse consequences for their child's social-emotional development.[9-12] However psychosocial intervention can be effective in improving the relations and quality of life after child abuse. [13-19]

The effects of child abuse differ, depending on the type of abuse. Childhood emotional and sexual abuse was strongly related to adult depressive symptoms, while exposure to verbal abuse and witnessing of domestic violence had a moderately strong association, and physical abuse a moderate one. For depression, more than two types of abuse exerted synergetically stronger symptoms. Sexual abuse was particularly harmful and caused symptoms of depression, anxiety, dissociation, mania and limbic irritability. Childhood verbal abuse had a stronger association with anger and irritability. The emotional abuse is related with dissociative symptoms. The risk factors of child abuse are multifactorial.[20] They are related to the environment, social values, culture, socioeconomic status, disability, death of mother or father or both, family trauma, lack of affection, parental disputes, repeated illness, violence, and aggression in behaviors. French INSEE survey, some statistically significant correlations were found between child abuse and risk factors like repeated illness and family trauma encountered by the child before the age of 18 years. [21]

According to Menahem G, the French sociologist who found out correlations by studying health inequalities, child abuse and its origins in the family, where it was associated with the lack of affection, parental discord, the prolonged absence of a parent, or a serious illness affecting either the mother or father. [22]

Children who were exposed to risk factors of child abuse and neglect were 59% more likely to be arrested as juveniles, 28% more likely to be arrested as adults, and 30% more likely to commit violent crime than children who were not exposed. [23-25]

Objectives

Primary Objective of this study was:

To find the determinants and risk factors of child abuse.

Secondary Objectives of this study were:

1. To find type of child abuse and severity of trauma related to child abuse.
2. To see the attitude of the families towards the child who suffered from child abuse.

Material and methods

Study design: Cross sectional survey

Setting: The study was conducted in ten Government Schools in Lahore city and the Center of Child abuse at Children hospital Lahore.

Duration: This study was completed in a year 2013.

Targeted population: Children aged <14 years.

Sampling Technique: Non probability Sampling; purposive sampling

Sample size: At 95% confidence interval and 80% power of study sample size was calculated as 300 by Epi info 2000. The sample size of 150 cases was drawn from 10 government schools form Lahore and 150 cases were selected from Center of Child Abuse Children Hospital Lahore.

Sample selection criteria

Inclusion: Children less than 14 years and any gender were included. They were abused sexually, physically, mentally or psychologically belonging to the lower, middle and upper class.

Exclusion: Children mentally and physically not stable. The parents refused to give consent to participate in research study.

1. Child Abuse Scale (CAS): screening device

The Child Abuse Scale comprised of 20 questions was used to identify children with different levels of abuse. The responses of the subjects were recorded on a 4-point rating scale. CAS score was determined as the sum of scores on each item describing trauma after child abuse that ranges from 34-136. Below 54 was mild, 55-65 as moderate and above 66 was considered as severe child abuse.

2. Demographic Questionnaire

A demographic form was also be developed to obtain personal information of the respondents like age, gender, birth order, socioeconomic status, family size, parental education and occupation, family system i.e. joint or nuclear.

Ethical consideration

Permission from the school authorities and management of Children hospital after explaining them the objectives of the study due to its critical nature was obtained. The participating children were approached with the help of their class teachers and were given informed consent before administrating the questionnaire comprising of Child Abuse Scale. Mother and father filled consent forms and bio-data forms. They were instructed to feel free if they had any queries.

Data collection tools: Questionnaire

Data Analysis

Data was entered and analyzed through SPSS 17. Mean±SD was calculated for quantitative variables while frequency and percentages were calculated for qualitative variables. Children were grouped as mild, moderate and severely abused on the basis of their CAS scores. Groups were compared by using chi-square test for qualitative variables and t-test for quantitative variables. P-value of <0.05 was considered as significant.

Results

The mean ages of the respondents in years were noted as 11.95 ± 1.68 years. According to the study results the maximum and minimum values of the ages in years of the respondents were 9 & 14 years respectively.

The study results showed that there were 11 (3.67%) respondents with the age of 9 years. Similarly 79 (26%) respondents were those who belonged to the age of 10 years, 44 (14.67%) respondents had age of 11 years, 29 (9.67%) respondents had age 12 years. There were 54 (18%) respondents who had age of 13 years and 83 (27.67%) respondents belonged to the age of 14 years.

Study results showed that there were 136 male respondents and these respondents were 45.33 percent in the total respondents. Similarly there were 164 females and these respondents were 54.67 percent in the total respondents.

The study results about the description of living area showed that there were 103 (34.33%) respondents were living in the rural area and 197 (65.67%) respondents were belonging to the urban area.

The results showed that 270 respondents were the abused person who belonged to the student category and those were in maximum number having 90 percent of the total respondents. There

were only 19 respondents who belonged to the labor category and those respondents are 6.33 percent of the total respondents. There were only 11 respondents who were belonging to the other occupations and these respondents were 3.67 percent of the total respondents.

The results of the study showed the education status of the respondent. There were 194 (64.67%) respondents belonging to the government school education, similarly there were only 11 (3.67%) those who were belonging to the private school education. The results showed that there were 48 (16%) respondents who were belonging to the traditional education, 36 (12%) respondents are those who belong to the Non Government Organization schools. The study results showed that there were only 11 (3.67%) respondents who were belonging to the other education system.

The study results showed that there were only 19 (6.33%) respondents who were smokers or drug addicts while 281 (93.67%) respondents were those who did not smoke or use drugs for addiction.

The study results showed that there were 50 (16.67%) respondents who were abused sexually, 197 (65.67%) respondents were abused physically, 43 (14.33%) respondents were abused emotionally and only 10 (3.33%) respondents were abused other reasons. There was significant difference between both genders (p-value = 0.000). Females were at more risk of having sexual abuse, while male children had more physical and emotional abuse.

The study results showed that there were 119(39.67%) respondents who faced an injury/ trauma after abuse. There are 38 respondents are belonging to the middle class status and these respondents are 12.67 percent of the whole data. Similarly there are 262 respondents who are belonging to the lower class status and these respondents are 87.33 percent of the whole respondents.

Description about the gender of abuser showed that there were 214(71.33%) respondents who were belonging to the male category while there were only 76(25.33) respondents who were belonging to the female category. The study showed that there were 10(3.33%) cases of child abuse who were abused by both genders. There were more males who abused the male respondent while more female respondent reported of being abused by females (p-value = 0.000).

The study results show that there were 261(87%) respondents who were threatened at the time of abuse. Similarly there were 29(9.67%) respondents who were in danger at the time of abuse. There were only 10(3.33%) respondents who were out of fear at the time of abuse. The inquiry about "have you told anyone about the abuser immediately after child abuse?" showed that there were 159(53%) respondents who responded as yes and there were 141(47%) respondents who responded that they did not tell about the abuser.

The study results showed that there were 8(2.67%) respondents who were stranger while the 8(2.67%) respondents were family friends. There were 7(2.33%) respondents were neighbor. There were 18(6%) respondents who were living in the same area. The results showed that there were 32(10.67%) respondents whose abuser were their uncle. There were 8(2.67%) respondents whose abuser were their mother's or father's friend. There were 36(12%) respondents whose abuser were their school teacher. The results showed that there were 22(7.33%) respondents whose abuser were their traditional teacher. The 7(2.33%) respondents whose abuser were their grandfather. The respondents related to the instructor abuser were 11(3.67%). There were 8(2.67%) respondents whose abusers were their real father and 39(13%), 42(14%) respondents belonged to their stepfather and stepmother respectively. The study results showed that there were 58(19.33%) respondents who were beaten by their mother and 48(16%) respondents were beaten by their father. Similarly there were 18(6%) & 41(13.67%) respondents were beaten by their brother, uncle and aunt. There are 45(15%) respondents who were beaten by other relatives. The 34(11.33%) respondents who were mostly physical abused by their teacher. There were 11(3.67%) respondents who were beaten by the employers. There are 47(15.67%) respondents who were slapped most of the time. There were 126(42%) respondents who were kicked. The study results showed that there were 50(16.67%) respondents who suffered bleeding after abuse.

The study results showed that there were only 11(3.67%) respondents who faced any physical disability and 289(96.33%) respondents who did not face physical disability after abuse.

The study results showed that there were only 43(14.33%) respondents who faced psychological problems after abuse. There were 63(21%) respondents whose mother had died There were 83(27%) respondents whose father had died. The study results showed that there were 228(76%) respondents who had their real mother while 72(24%) respondents had their stepmothers.

The study results showed that there were 205(68.3%) respondents who had their real father while 95(31.7%) respondents have their stepfathers.

The results showed that there were 100 (33.33%) respondents who had 1 sibling and 93 (31%) respondents who had 2 siblings. There were 36 (12%) respondents who had 3 siblings similarly 39 (13%) respondents who had 4 siblings. There were 22 (7.33%) respondents who had 5 siblings. There were 10 (3.33%) respondents who had 6 ore more siblings.

There were 172 (57.33%) respondents who were living with their real parents while 97 (32.33%) respondents who were living with stepfather and 20 (6.67%) living with stepmother. There were 11 (3.67%) respondents who were living in hostels/ institutions.

There were 11 (3.67%) respondents whose father were businessmen and 35 (11.67%) respondents whose father were clerk. There were 22 (7.33%) respondents whose father were working in factory while 8 (2.67%) respondents father were hawker. The 71 (23.67%) respondent's father was in labor category and the 100 (33.33%) respondent's fathers were shopkeepers. There were 21 (7%) respondents whose fathers were teachers.

There were 10 (3.33%) respondents whose mothers were the working women while the rest of 290 (96.67%) were house wives.

There were 60 (20%) respondents whose fathers were illiterate. There were 11 (3.67%) respondents whose mothers were graduate while 269 (89.67%) whose mothers were illiterate.

Discussion

Sexual abuse of children refers to sexual behavior between a child and an adult or between two children when one of them is significantly older or uses coercion. The sexual behaviors included touching breasts, buttocks, and genitals whether the victim was dressed or undressed and penetration of the vagina or anus with sexual organs or with objects. Pornographic photography was included in the definition of sexual abuse. [26]

According to our survey, the mean age of the respondents was 11.95 ± 1.68 years. It was observed that there were 136 (45.33%) males and 164 (54.67%) females in the study with male to female ratio of 1: 1.2. This showed that female children were at more risk of being abused. According to literature evidences, female gender was at more risk of abuse (16.8%) as compared to men or male gender (7.9%). Number of substantiated or indicated cases had decreased by 41% in the time period of 1992 to 2000. [27] Another study reported that approximately 15% to 25% of women and 5% to 15% of men were sexually abused when they were children. [28]

Our survey results showed that there were 90 (30%) respondents were of age 9-10 years, 73 (24.33%) respondents who have age of 11-12 years and there were 137 (45.67%) respondents had age of 13-14 years. Literature also showed that the incidence of child sexual abuse increased with age like: 0-3 years 10% of victims, 4-7 years 28.4% of victims, 8-11 years 25% of victims and 12 and older 35.9% of victims. [29]

According to our survey, there were 8(2.67%) abusers were stranger while the 8(2.67%) abusers were family friends, 25(8.33%) were neighbor or living in the same area, 40(13.33%) were abused by their uncle or mother's or father's friend. 68(22.67%) were abused by school or madrisa teacher. There are 8(2.67%) respondents whose abusers were fathers and 39(13%), 42(14%) respondents belong to their stepfather and stepmother respectively. Research showed that most sexual abuse offenders were acquainted with their victims; approximately 30% were relatives of the child, most often brothers, fathers, mothers, uncles or cousins; around 60% were other acquaintances such as friends of the family, servants, or neighbours; strangers were the offenders in approximately 10% of child sexual abuse cases. [30]

According to our survey, there were 50 (16.67%) respondents were abused sexually, 197 (65.67%) were abused physically. This survey showed that female children were at more risk of having sexual abuse, while male children had more physical and emotional abuse. Among fatal cases, the major-

ity suffered from physical abuse (16.1%), sexual abuse (9.1%), psychological maltreatment (7.3%), or a combination. Sexual and physical abuse rates increased with age, with the exception of violence towards infants aged less than 1 year. More than 33% of fatalities were associated with multiple forms of maltreatment. [31]

Children who were physically abused were likely to receive bone fractures, particularly rib fractures.[23] The study results showed that there were 119(39.67%) respondents who faced an injury/ trauma after abuse. Risk increased for those with physical disabilities, especially those that impaired the child's perceived credibility: blindness, deafness, and mental retardation. Boys were over represented among sexually abused children when compared to sexually abused children without disabilities.[32]

Data showed that 87.3% abuser belonging to the lower class status and there were more male abusers 214 (71.33%) involved and mostly victims were threatened by the abuser at the time of abuse 261 (87%) and mostly children did not tell about this to any one (47%). Risk factors for maltreatment included medical problems such as mental retardation, emotional disturbance, visual or hearing impairment, learning or physical disabilities, and behavioral problems. [33] Children having absence of one or both parents, presence of stepfather in home doubles the risk for girls and parental impairments are also associated with increased risk. [31]

In symptomatic abused children, 62.8% qualify for at least one psychiatric diagnosis, 29.5% qualify for 2 or more psychiatric diagnoses. Cognitive behavioral therapies were affective for some symptoms of child sexual abuse and appropriately treat for psychiatric diagnoses. [33]

Conclusion

Thus it was concluded form this survey that females were at more risk of being abused, whether sexually or physically. Parents and teachers were required to give more attention to their children and students. The health of children and communities should be protected by health education, public awareness and psychological therapies against child abuse.

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Cardiovascular risk profile in patients with pre-diabetes

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Abstract

Background: Although it is well known the effect of diabetes on cardiovascular risk there still exists controversies about the importance of pre-diabetes on the incidence of cardiovascular diseases in this population.

Objective: We aimed to determine the differences in cardiovascular risk factors between normoglycemic and pre-diabetic patients.

Method: This is a cross-sectional study including 211 participants aged more than 20 years who underwent an oral glucose tolerance test with 75 grams of glucose. Diabetic patients were excluded.

Results: Mean age was 51.5 years and 67.78% were women. Fasting plasma glucose was normal in 47.4% and impaired fasting glucose was present in 52.6%. According to the both fasting and 2-hour OGTT, 47.9% of participants had pre-diabetes (IFG and/or IGT), 39.8% of participants had normal glucose levels, and, 12.3% of participants had diabetes. Both IFG and IGT were found in only 17.5%. HDL-c was significantly lower in patients with pre-diabetes than in those with normoglycemia: 45.7 (SD 10.6) mg/dl vs 49.8 (SD 9.9) mg/dl, p=0.009). TG and TG/HDL-c levels were significantly higher in patients with pre-diabetes than in the normoglycemic group (TG: 150.7 (SD 97.6) mg/dl vs 125.9 (SD 58.4) mg/dl, p=0.049, and TG/HDL-c; 3.4 (SD 1.9) vs 2.7 (SD 1.6), p=0.012 respectively). HbA1c levels were higher in pre-diabetic patients (6.0 (SD 0.4)% vs 5.6 (SD 0.3)%, p=0.041). Blood pressure levels were also worse controlled in pre-diabetic patients than in normoglycemic ones (38.6% vs 66.7%, p<0.001).

Conclusion: In our study patients with pre-diabetes had major risks for coronary artery disease. Since pre-diabetes is a critical stage, to properly identify risk factors could reduce the development of the cardiovascular diseases.

Key words: Pre-diabetes, cardiovascular diseases, risk factors

Introduction

Diabetes mellitus (DM) is one of the most important public health problems. Data from World Health Organization shows that it affects at least 347 million people worldwide (1). The prevalence of DM has been increasing significantly during the last years (2). TURDEP Study showed that 2.6 million people in Turkey had diabetes and over 2.4 million had impaired glucose tolerance in the year 2000 (3). In Europe in the year 2011, almost 53 million had diabetes, being the prevalence especially higher in the eastern European countries (4).

DM has a negative effect on the quality of life of patients with complications and causes a high costs both at the individual and population levels (5). Therefore, precautions should be taken to early diagnose DM because that has shown to be effective in reducing the consequences of this disease (6). Macrovascular effects, one of the most important complications of DM, are the responsible for the higher incidence of cardiovascular diseases (CVD) (7). This higher risk of CVD and mortality has been scored by measuring daily physical activity, intake of fruits and vegetables, body mass index (BMI), waist circumference, plasma glucose level and the presence of family history of diabetes (8,9). Pre-diabetes is an early stage in the progression towards DM and has been defined as the presence of impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) (10). Several studies have shown an increasing trend in the prevalence of this condition, rising up to 75% of adult Americans or 15% of Chinese population (11).

Some studies have shown that these patients could also have a higher cardiovascular risk (12,

13). Pre-diabetes can overlap with obesity, dyslipidemia (high triglycerides and/or low high-density lipoprotein) and hypertension, which eventually means an increase of coronary risk A recent study from Turkey pointed out a prevalence of pre-diabetes of nearly 30% among Turkish people (14), but we have no information about the association between pre-diabetes and cardiovascular risk factors. Regarding this question we designed a study aimed at knowing whether pre-diabetes could be associated with a higher prevalence of cardiovascular risk factors.

Methods

This is a cross-sectional study in which a sample of participants undergoing a test of oral glucose tolerance test (OGTT) was analyzed. Participants were consecutively selected from an internal medicine clinic in a general Turkish academic hospital. Patients were asked to participate after explaining them the objectives of the study and those who did not want to participate were excluded. During a period of six months a total of 211 of participants were-included in the study. The results of the clinical and laboratory analysis were delivered to the patients and the doctors responsible for their care.

All participants underwent an OGTT test. During the test, patients for at least 8 hours of fasting (optimal 8-14 hours of fasting) came in the morning without smoking, not to take food or drink other than water are proposed and tested in a quiet room. Samples of blood were taken initially (0. minutes), and 2 hours after taking 75 grams of glucose. Classification of patients depending of the glucose levels was made following the recommendations of the American Diabetes Association criteria (15). Participants were divided into three groups: according to these criteria: fasting plasma glucose 100-125 mg/dL and/or 140-199 mg/dL after OGTT were classified as pre-diabetes, fasting plasma glucose ≥126 mg/dL or 2-hour post-OGTT plasma glucose level of ≥200 mg/dL as diabetes, and fasting plasma glucose <100 mg/dL and 2-hour post-OGTT glucose value <140 mg/dL as normoglycemic. We excluded those having FPG ≥126 mg/dL and/or 2-hour post-OGTT glucose level ≥200 mg/dL, previous known diabetes, patients taking anti-diabetic medication, steroids or drugs affecting the glucose metabolism, pregnant women and those with malignancy or acute disease.

Patients' age, gender, family history of diabetes, height, weight, FPG, 2-hour post-OGTT glucose values, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, triglycerides (TG) and blood pressure (BP) levels were recorded. BMI, and TG/HDL-c ratios were calculated. Both, pre-diabetic and normoglycemic patients were also assessed for glycosylated hemoglobin (HbA1c) levels. These levels were considered normal if they were lower than 6.1%.

BMI was defined in three categories: normal, overweight and obesity depending of body mass index was lower than 25 kg/m², 25 to 29.9 kg/m² and ≥30 kg/m², respectively. HDL cholesterol was considered bad controlled if lower than 39 mg/dL in women and lower than 35 mg/dL in men, TG was bad controlled if higher than 150 mg/dL as proposed by World Health Organization (16). According to the Joint National Committee (JNC) 7 the normal value for blood pressure was fixed in 140/90 mm Hg (17).

Statistical power: The results of our study allow us to have a 94% statistical power, accepting an alpha risk of 0.05 in a two-sided test with 84 subjects in the first group and 101 in the second, to recognize as statistically significant the difference.

Statistical analysis: An initial data analysis to validate the data entered was performed. To assess the comparability of baseline characteristics between study groups, chi-square test (or Fisher's exact test) for categorical variables and Student's t test (or the Mann-Whitney test) for continuous variables were used. Multivariate logistic regression models were fitted to avoid the effect of potential confounders. For this analysis, the estimations were adjusted by baseline factors such as demographic and predictive factors for the other variables. As a sensitive analysis, unadjusted estimates also were performed in different scenarios to assess the robustness of the results. The 95% confidence interval was estimated for all parameters. Statistical analysis was performed using the Statistical Package for Social Sciences SPSS v17 and the level of significance will be set to 5%.

Results

A total of 211 cases were examined. Mean age was 51.5 (SD 13.3) years and 67.78% were women. According to fasting plasma glucose levels 47.4% of participants (n=100) were normal and 52.6% of participants (n=111) had impaired fasting glucose. According to the both fasting and 2-hour OGTT glucose levels 47.9% of participants (n=101) had pre-diabetes (IFG and/or IGT), %39.8 of participants (n=84) had normal glucose levels, and, %12.3 of participants (n=26) had diabetes. Both IFG and IGT were found in only 17.5% (n=37). Figure 1 shows in a flow chart the distribution of participants according the procedures and the results of the blood samples. It was seen whether the participants did not undergo OGTT, the diagnoses of 16% of them previously classified as normoglycemic by fasting plasma glucose (%14 IGT, %2 DM) could have been misleading.

Pre-diabetic participants were older and had significantly higher blood pressure. Lower HDL-c figures in persons with pre-diabetes than in those with normoglycemia were found. Besides, the ratio TG/HDL-c was also worse controlled in pre-diabetic persons (Table 1). HbA1c levels were significantly higher in pre-diabetic participants (p=0.041). Assessment by HbA1c, only %14 pre-diabetics had lower than 6.1%. We did not found differences related to sex or in the rest of variables analyzed. Characteristics of patients, which have IFG or IGT or both have shown in table 2. Multivariate analyses confirmed the relationship between pre-diabetes and higher levels of HbA1c (OR 4.77, 95% CI 2.03 to 11.21) and lower levels of HDL-c (OR 0.95, 95% CI 0.92 to 0.99) (Table 3). The effect of the bad control of blood pressure on pre-diabetes prevalence was on the limit of the significance OR 1.97 (95% CI 0.99 to 3.90, p=0.05).

Discussion

This study shows that patients with pre-diabetes had a high risk for cardiovascular diseases even before being diagnosed from diabetes.

It has been pointed out that a number of commonly measured clinical variables as a risk score (including body mass index, systolic blood pressure, family history of diabetes, ethnicity, age, sex, fasting glucose, and lipids) may have a better

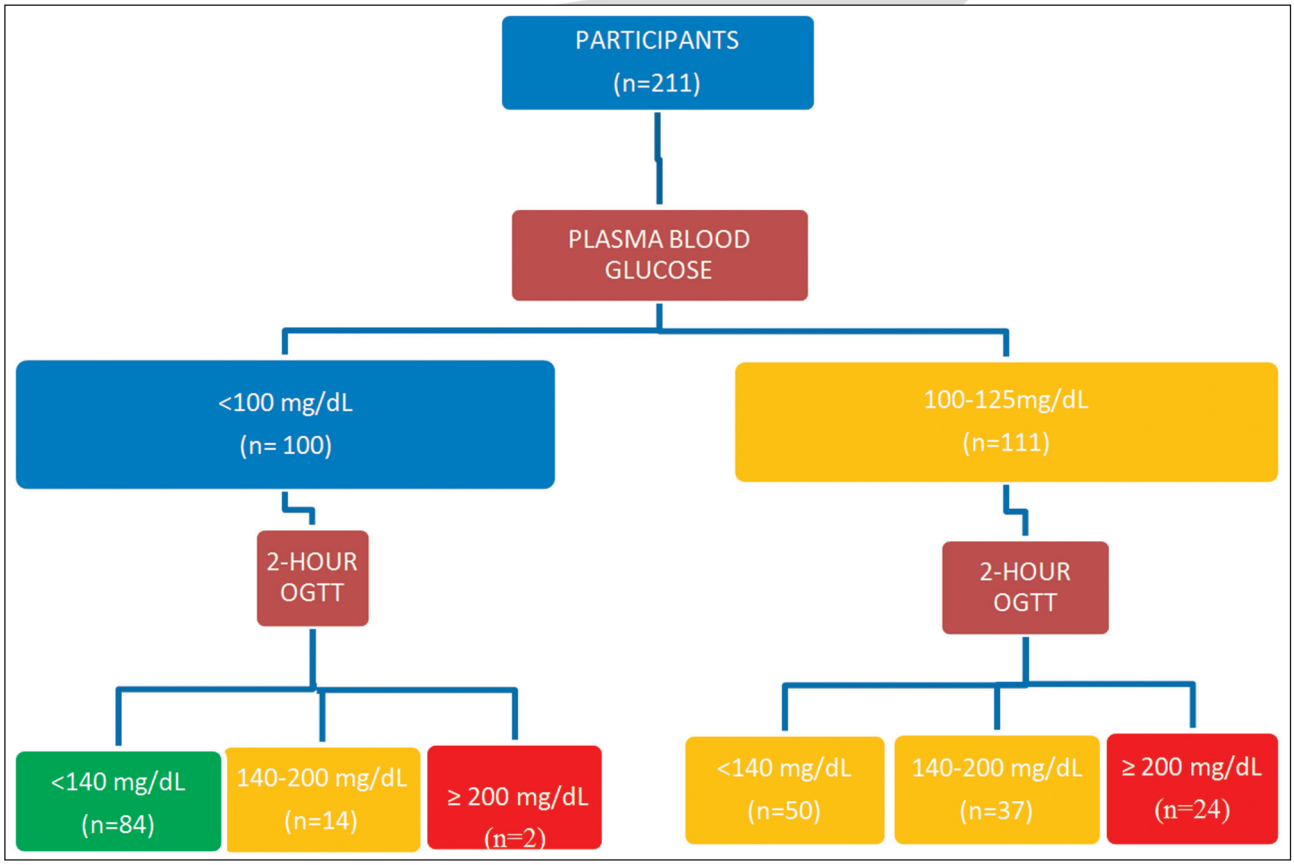

Figure 1. The distribution of participants according the procedures and the results of the blood samples

Table 1. Characteristics of the patients according to the results of oral glucose tolerance test (values are showed in percentages except age)

	Pre-diabetic N=101	Normoglicemic N=84	P Value
Age (mean, SD) years	53.6 (14.0)	47.4 (12.2)	0.002
Sex (women)	63.4	71.4	0.245
History of diabetes	45.5	46.4	0.904
Medication for blood pressure	35.6	20.2	0.021
Medication for Lipids	19.8	2.4	<0.001
Control of cardiovascular risk factors (good control)			
Body mass index (BMI)			
<25 kg/m^2	18.8	34.5	(p-trend)
25-29 kg/m^2	45.5	33.3	
≥30 kg/m^2	35.6	32.1	0.092
Obesity (BMI ≥ 30) %	35.6	32.1	0.617
HDL[a]-control (>39 mg/dL in women or >35 mg/dL in men) %	39.6	58.3	0.011
Triglycerides <150 mg/dL %	62.4	71.4	0.194
Blood pressure <140/90 mmHg %	38.6	66.7	<0.001
HbA1c <6.1%) % [b]	14.3	48.5	<0.001
Continuous values of cardiovascular risk factors (mean, SD)			
Triglycerides /HDL-c	3.42 (1.93)	2.74 (1.68)	0.012
Body mass index (kg/m^2)	29.15 (4.89)	27.84 (5.17)	0.09
HDL-c (mg/dL)	45.76 (10.67)	49.80 (9.91)	0.009
Triglycerides (mg/dL)	150.70 (97.60)	125.90 (58.43)	0.049
HbA1c (%)	6.04 (0.43)	5.61 (0.32)	0.041
Total cholesterol (mg/dL)	208.58 (38.57)	210.07 (38.88)	0.795
Systolic blood pressure (mm Hg)	130.89 (17.83)	123.21 (17.08)	0.003
Diastolic blood pressure (mmHg)	84.75 (11.45)	80.00 (11.19)	0.005

[a] HDL-c: High Density Lipoprotein Cholesterol; [b] HbA1c: Glycosylated hemoglobin.

Table 2. Characteristics of patients depending on they have Impaired Fasting Glucose (IFG) or Impaired Glucose Tolerance (IGT), and both (this table include all patients before oral glucose tolerance test)

	IFG (N=111)	Normal (N=84)	p	IGT (N=51)	Normal (N=84)	p	IFG and IGT (N=37)	Normal (N=84)	p
Age (mean, SD) years	53.9 (12.5)	47.4 (12,2)	<0.001	57.2 (14.2)	47.4 (12,2)	<0.001	54,2 (13,4)	47.4 (12.2)	<0.001
Sex (women)	65.8	71	0.400	66.7	71.4	0.560	70.3	71.4	0.897
History of diabetes	49.5	46.0	0.666	45.1	46.4	0.880	48.6	46.4	0.822
Medication for Hypertension	40.5	20.2	0.003	45.1	20.2	0.002	48.6	20.2	0.001
Medication for Hyperlipidaemia	21.6	2.4	<0.001	23.5	2.4	<0.001	24.3	2.4	<0.001
Control of cardiovascular risk factors									
Body Mass Index									
<25 kg/m^2	13.5	34.5	p trend (0.011)	23.5	34.5	p trend (0.467)	16.2	34.5	p trend 0.301
25-29 kg/m^2	47.7	33.3		45.1	33.3		54.1	33.3	
≥30 kg/m^2	38.7	32.1		31.4	32.1		29.7	32.1	
HDL-c[a] (>39 mg/dL in women or >35 mg/dL in men)	32.4	61.0	<0.001	41.2	58.3	0.053	29.7	58.3	0.004

Triglycerides <150 mg/dL)	58.6	60.0	0.064	56.9	71.4	0.083	54.1	71.4	0.063
Blood Pressure (<140/90 mmHg)	31.5	64.7	<0.001	33.3	66.7	<0.001	29.7	66.7	<0.001
HbA1c (<6.1%)[b]	46.8	85.7	<0.001	45.1	85.7	<0.001	40.5	85.7	<0.001
Continuous values of cardiovascular risk factors (mean, SD)									
Triglycerides (mg/dL)	149.6 (78.8)	125.9 (58.4)	0.022	151,9 (56,3)	125,9 (58,4)	0.012	156.2 (59.7)	125,9 (58.4)	0.010
Triglycerides /HDL-c	3.3 (3.2)	2.2 (1.6)	0.007	3,0 (1.8)	2,2 (1,6)	0.009	3,1 (3.0)	2,2 (1.67)	0.002
Total cholesterol (mg/dL)	207.0 (38.1)	210.0 (38.8)	0.591	209,8 (41,7)	210,07 (38.8)	0.972	207.2 (39.1)	210,0 (38.8)	0.709
HDL-c (mg/dL)	44,4 (9.8)	49,8 (9.9)	<0.001	46,2 (11,4)	49,81 (9,91)	0.055	43.7 (9.8)	49.8 (9.9)	0.002
Body Mass Index	29.1 (4.9)	27.4 (5.1)	0.024	28.5 (5.4)	27.4 (5.1)	0.234	29.1 (5.48)	27.4 (5.1)	0.113
Systolic Blood Pressure(mmHg)	134.2 (17.8)	123.2 (1 (7.0)	<0.001	132,1 (18,5)	123,2 (17,0)	0.005	133,8 (16.7)	123,21 (17.0)	0.002
Diastolic blood pressure (mmHg)	86.5 (10.9)	80.0 (11.19)	<0.001	86,2 (11.4)	80.0 (11.19)	0.002	86,3 (9.5)	80,0 (11.1)	0.003

[a]HDL-c: High Density Lipoprotein Cholesterol; [b] HbA1c: Glycosylated hemoglobine

Table 3. Odds ratio for having pre-diabetes, according to the cardiovascular risk factors, adjusted by age and sex

	Odds Ratio	95% Confidence interval
HbA1c [a]	4.77	2.03 to 11.21
HDL-c[b]	0.95	0.92 to 0.99
Blood pressure <140/90 mmHg	1.97	0.99 to 3.90
Sex (women)	0.88	0.42 to 1.88
Age (years)	1.01	0.99 to 1.04

[a] HbA1c: Glycosylated hemoglobin; [b]HDL-c: High Density Lipoprotein Cholesterol.

ability for diabetes and considerably greater cardiovascular disease prediction (18,19). Since pre-diabetes is an early stage for diabetes we focused on most of these parameters in this study to determine if even before developing DM, cardiovascular risk was worse in these population.

Among cardiovascular risk factors assessed in our study, patients with pre-diabetes had worse levels of HDL, TG/HDL-c levels and blood pressure controlling than normoglycemic ones. Both total cholesterol and LDL cholesterol obtained in our sample were slightly higher than those obtained in general population in Turkey (20), which is expected, since we have studied a population at risk. Nevertheless the differences were scarce.

There are some other studies that give the relationship between the different numbers of components of CHD. Wu et al has showed a relation between worsening blood pressure and pre-diabetes as we found (21). What is especially important given the fact that their combined presence could have as a consequence a more severe coronary artery disease (22). ***A study*** by Alexander et al. (2006) demonstrated a progressive increase in waist circumference, reduced HDL cholesterol, elevated triglycerides, and elevated blood pressure as glycemia worsens from normal through IFG, IGT and diabetes (23).

We also studied total and LDL cholesterol, but the levels of these components between the two populations were not significant. Because it is well known that HbA1c is a risk factor itself and that a continuous relationship between FPG and HbA1c related to the cardiovascular risk has been shown (24) we compared the differences in HbA1c between pre-diabetics (including both FPG and IGT) and normoglycemic patients. As it was expected, we found higher levels of HbA1c

in the first ones. This finding also contributes to support the idea of being more careful in the management of this group of patients.

Some studies claimed the usefulness of HbA1C as a diagnostic criteria for classifying patients in pre-diabetic stage (24, 26).

However we found that a number of diagnoses of pre-diabetes can be missing by using only that method, as well as the study of Olson et al supported (27). Hence, the use of the proposed HbA1c criteria would not be a good screening strategy with our findings.

In the United States, it has been found that each year, up 11% of persons with pre-diabetes who do not lose weight and do not do moderate physical activity will progress to type 2 diabetes (28). IFG an IGT have been increasingly debated in some studies on whether the benefits of pharmacologic treatments with lifestyle change interventions in these early stages could be useful to prevent the development of the disease and the cardiovascular complications (12,29,30). Meanwhile, lifestyle modification aside, medication in pre-diabetic individuals is still individualizing. According the results of our study, we believe that pharmacologic glucose-lowering medications in pre-diabetes with other risk factors could be appropriate.

Limitations and Strengths of Our Study

Participants came from patients attending to a hospital consults which can differ with general population. On the other hand, the cross-sectional design of the study does not allow us to infer causal relationships but helped to us to generate hypothesis to be tested in further studies. It would be interesting to carry out a prospective study to know if, in this population, differences in cardiovascular profile can be followed by a higher percentage of cardiovascular events.

All laboratory tests were collected in the same center and the main researcher took all the data from the patients, which reduced the variability of the measures.

This is the first study showing cardiovascular risk in pre-diabetic patients in Turkey, a country with a high ethnic and geographic variability.

Conclusion

Patients with pre-diabetes have a higher risk for cardiovascular diseases. Screening, identifying the risk factors and taking precautions earlier would reduce the development of the cardiovascular heart disease yet during the period of pre-diabetes.

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Knowledge, beliefs, and Practices on Type 2 Diabetes and its Treatment among Saudi Patients

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Abstract

Objective: was to measure diabetic patients' knowledge about type 2 diabetes, its complications and to identify their beliefs regarding its predisposing factors, together with identifying their practices on aspects related to self-care management.

Methods: Cross–sectional survey was conducted. Convenient method of sampling was adopted. The study included all adults (≥18 year) type 2 diabetic patients who received medical care in diabetic centers in hospitals and primary health care settings in the Western Region of Saudi Arabia.

Results: Overall 670 patients were participated in this study. Of them (61%) were classified as knowledgeable about the disease. The level of patients' knowledge was found to be significantly associated with patients' educational level ($P<$ 0.001) and residence (P = 0.011). Out of all patients (88.2%), (82.5%) and (79.2%) agreed that obesity, lack of physical activity, and family history are predisposing factors to diabetes respectively. Nearly 40% of the patients admitted that they did exercise on a regular basis and half of them visited their doctors for routine check–up. More than 50% of the participants used to monitor their blood glucose level at homes.

Conclusion: In conclusion the results of the current survey showed a gap in patients' knowledge on diabetes and its complications. Patients should be empowered through the provision of continuous educational program with special emphasis on illiterates, less educated and rural residents.

Key words: Type 2 diabetes, knowledge, practice, Saudi Arabia

Introduction

Diabetes mellitus, particularly type II, is a major public health concern worldwide. In the next two decades there will be an alarming increase in the population with type II diabetes mellitus all over the world [1]. Saudi Arabia has one of the highest prevalence rates of diabetes worldwide[2]. Diabetes is a complex disorder that requires constant attention to diet, exercise, glucose monitoring, and medication to achieve good glycemic control[3]. Uncontrolled diabetes may be associated with serious medical complications leading to increase in premature morbidity and/or mortality[4]. Regular exercise has important effects on controlling hyperglycemia and on the prevalence of cardiovascular complications in type II diabetic patients[5]. Helping patients to gain knowledge, skills, resources and support are essential for optimal health and can assist in early detection of the disease and reduce the incidence of complications[6].

Many studies were conducted a round the world to understand the knowledge, attitude and practices (KAP) regarding diabetes mellitus among diabetic patients. Knowledge about diabetes was found to be proportionally increased as the duration with the disease increased[7]. Hue *et al.*[8] found that half of diabetic patients did not adhere to regular exercise and diet and there was no correlation between the knowledge and practice of diabetic patients and their blood glucose control. Maina *et al.*[9] studied the KAP of diabetes; on average, they found that nearly three quarter of the respondents had poor knowledge and practices about the disease. Assessment of KAP of type II diabetic patients by Badruddin *et al*.[10] showed overall satisfactory awareness about the risk of diabetes complications, but the misconceptions regarding diet, insulin and diabetes were quite common. In Saudi Arabia researchers identified good knowledge but poor attitude and practices towards diabetes among female patients[11].

Understanding patients' knowledge, beliefs, and practices towards diabetes, its complications, and treatment is crucial for the provision of standard care. This study was conducted to measure diabetic patients' knowledge about the diseases,

its complications and to identify their beliefs regarding its predisposing factors, together with identifying their practices on aspects related to self-care management.

Methods

Study design

A cross–sectional survey was conducted during six months period (June – November 2013).

Settings and Participants

The study included all adults (≥18 year) type 2 diabetic patients who received medical care in diabetic centers in hospitals and primary health care settings in the Western Region (Jeddah, Mekkah, and Taif), Saudi Arabia. Newly diagnosed diabetic patients and patients refused to participate in the study were excluded.

Sample size and sampling technique

Convenient method of sampling was adopted, whereby 670 patients were recruited.

Data collection

Data was collected by trained pharmacy students through face-to-face interviews using structured questionnaire after obtaining verbal informed consent from the participants. The questionnaire was developed by the research team after thoroughly searching the relevant literature. The questionnaire was designed to collect data on patients' demographics and their knowledge, beliefs and practices towards diabetes. Patients' knowledge was assessed through 15 questions related to the disease, its complications, role of medical treatment and exercise in controlling blood glucose level. Responses in this section were recorded as "Yes", "No", and "Don't know". Scoring system was developed and a cut-off point (median score) was determined to measure patients' knowledge. Accordingly, patients were classified as either had good knowledge (scored ≥ median) or poor knowledge (scored< the median). Patients attitude on predisposing factors to diabetes were assessed using a 5- point–likert scale (Strongly agree, Agree, Neutral, Disagree, Strongly disagree). The last part of the questionnaire was designed to assess patients' practices related to lifestyle modifications and activities used in self-management of diabetes. Responses in this part were recorded as "Yes", "Sometimes", and "No". The questionnaire was tested with a group of 25 patients to ensure applicability.

Ethical approval for the study was obtained from Pharmacy Practice Research Unit (PPRU) ethical committee, College of pharmacy, Taif University.

Data analysis

Data was processed using Statistical Package for Social Sciences (SPSS) version 18. Descriptive statistics was used to describe all variables. Association between patients' knowledge score and their background characteristics was tested by Mann-Whiteny U test and Kruskal–Walis tests when appropriate. Chi-square test was used to determine association between patients' background characteristics and both patients' attitudes and practices. The level of $P< 0.05$ was considered statistically significant.

Results

Patients' background characteristics:

Overall 670 patients with a mean age (± SD) 51.7± 15 years were participated in the study. Females constituted406 (60.6%) and 584 (87.2%) of them were from urban. Nearly one quarter of the interviewed patients had higher education and 193(28.8%) were employees. Above 40% (288) had diabetes for > 10 years and 372 (55.5%) had one or more coexisting illness. Background characteristics of patients by gender type were presented in Table 1.

Patients' knowledge

A total of 171 (25.5%) patients correctly defined diabetes and more than three quarter (n=505) believed that the disease is inherited. Out of all patients 590 (88.1%), 567 (84.6%), 453 (67.6%) knew that diabetes can cause visual problems, complications on feet, and renal problems respectively. Out of the participated patients 279 (41.6%) and 207 (30.9%) claimed that diabetes associated with hepatic and pulmonary complications respectively. Considerable number 479 (71.5%) of participants knew diabetes complications are preventable and 529 (79%) considered that medical treatment alone cannot totally cure the disease. A

Table 1. Patients' background characteristics by gender type

Background characteristic	Gender		Total	P value
	Male	Female		
Age in years				
< 40	51(34.5%)	97(65.5%)	148	
40-65	156(39.2%)	242(60.8%)	398	0.153
>65	57(46.0%)	67(54.0%)	124	
Residence				
Urban	250(42.8%)	334(%57.2)	584	0.000
Rural	14(16.3%)	72(83.7%)	86	
Educational level				
University	86(53.1%)	76(46.9%)	162	
Secondary	56(50.5%)	55(49.5%)	111	
Intermediate	45(53.6%)	39(46.4%)	84	0.000
Primary	44(44.0%)	56(56.0%)	100	
Illiterate	33(15.5%)	180(84.5%)	213	
Employment status				
Employed	124(64.2%)	69(35.8%)	193	
Unemployed	46(12.9%)	310(87.1%)	356	0.000
Retired	94(77.7%)	27(22.3%)	121	
Duration of disease in years				
< 1	22(33.8%)	43(66.2%)	65	
1-5	58(39.5%)	89(60.5%)	147	0.000
6-10	59(34.7%)	111(65.3%)	170	
>10	125(43.4%)	163(56.6%)	288	
Co-morbidity				
Yes	136(36.6%)	236(63.4%)	372	0.054
No	128(43.0%)	170(57.0%)	298	

Table 2. Patients' responses on knowledge of diabetes and its treatment

Item	Yes	No	Don't know
Definition of diabetes	171(25.5%)	198(29.6%)	301(44.9%)
Diabetes is infectious disease	8(1.2%)	597(89.1%)	65(9.7%)
Diabetes is inherited disease	505(75.4%)	66(9.9%)	99(14.8%)
Diabetes can cause renal problems	4453(67.6%)	56(8.4%)	161(24.0%)
Diabetes can cause visual problems	590(88.1%)	20(3.0%)	60(9.0%)
Diabetes can cause CNS problems	224(33.4%)	163(24.3%)	283(42.2%)
Diabetes can cause hepatic problems	279(41.6%)	150(22.4%)	241(36.0%)
Diabetes can cause complications on feet	567(84.6%)	35(5.2%)	68(10.1%)
Diabetes can cause pulmonary problems	207(30.9%)	192(29.1%)	268(40.0%)
Diabetes complications are preventable	479(71.5%)	46(6.9%)	145(21.6%)
Medical treatment totally cure diabetes	27(10.7%)	629(79.0%)	69(10.3%)
Exercise reduce or control blood glucose level	552(82.4%)	24(3.6%)	94(14.0%)
Diabetic foot ulcer take longer time to heal	478(71.3%)	122(18.2%)	70(10.4%)
Diabetic patients need to recurrently visit ophthalmologist	525(78.4%)	65(9.7%)	80(11.9%)
Normal blood glucose level	387(57.8%)	164(24.45%)	119(17.8%)

Table 3. Associations between patients' knowledge and their background characteristics

Background characteristic	Total knowledge		Total	P value
	Good	Poor		
Gender				
Male	154(58.3%)	110(41.7%)	264	0.339
Female	255(62.8%)	151(37.2%)	406	
Age in years				
< 40	89(60.1%)	59(39.9%)	148	0.676
40-65	248(62.3%)	150(37.7%)	398	
>65	72(58.1%)	52(41.9%)	124	
Residence				
Urban	369(63.2%)	215(36.8%)	584	0.011
Rural	40(46.5%)	46(53.5%)	86	
Educational level				
University	116(71.6%)	46(28.4%)	162	0.000
Secondary	78(70.3%)	33(29.7%)	111	
Intermediate	43(51.2%)	41(48.8%)	84	
Primary	63(63.0%)	37(37.0%)	100	
Illiterate	109(51.2%)	104(48.8%)	213	
Employment status				
Employed	123(51.2%)	70(36.3%)	193	0.503
Unemployed	210(59.0%)	146(41.0%)	356	
Retired	76(62.8%)	45(37.2%)	121	
Duration of disease in years				
< 1	36(55.4%)	29(44.6%)	65	0.760
1-5	89(60.5%)	58(39.5%)	147	
6-10	107(62.9%)	63(37.1%)	170	
>10	177(61.5%)	111(38.5%)	288	
Co-morbidity				
Yes	233(62.6%)	139(37.4%)	402	0.091
No	176(59.1%)	122(40.9%)	268	

Table 4. Patients' beliefs on diabetes predisposing factors

Predisposing factor	Strongly agree	Agree	Neutral	Disagree	Strongly disagree	Significance & P value
Obesity	397(59.5%)	194(29.0%)	47(7.0%)	29(4.3%)	3(0.4%)	
Lack of physical activity	336(50.1%)	217(32.4%)	66(9.9%)	42(6.3%)	9(1.3%)	Age (P=0.021)
Family history	325(48.5%)	206(30.7%)	80(11.9%)	49(7.3%)	10(1.5%)	Educational level (P=0.006)
Stress	295(44.0%)	188(28.1%)	114(17.0%)	57(8.5%)	16(2.4%)	Educational level (P=0.046)
Increased sugar intake	323(48.2%)	199(29.7%)	76(11.3%)	42(6.3%)	30(4.5%)	Employment status (P=0.005)

higher percentage 552 (82.4%) of them knew the benefit of exercise in reducing or controlling blood glucose level. Patients' knowledge was shown in Table 2.

A total 409 (61%) respondents were classified as knowledgeable about diabetes, while 261 (39%) had poor knowledge. The level of patients' knowledge was found to be significantly associated with patients' educational level ($P< 0.001$) and residence ($P= 0.011$) as presented in Table 3.

Patients' attitude on diabetes predisposing factors

Out of all patients 591 (88.2%), 553 (82.5%) and 531 (79.2%) agreed that obesity, lack of physical activity, and family history are predisposing factors to diabetes respectively. However 522 (77.9%) and 483 (72%) participants respectively, attributed the onset of diabetes to increased sugar intake and stress, Table 4.

Patients' practices towards the disease and its management

Nearly 265 (39.5%) of the patients admitted that they did exercise on a regular basis and 334 (50%) of them visited their doctors for routine check–up. Regarding diet control; 255 (38.1%) had a diet plan at home and 146 (21.8%) admitted that they stick to this plan but, taking meals outside home. More than half 346 (51.6%) of the participants had used to monitor their blood glucose level at home. Table (5) shows patient practices correlated to different age groups.

Association between patients' knowledge and practices

Patients with good knowledge regularly were used to check their blood glucose level at home245 (59.5%) more than those with poor knowledge 101 (38.7%), ($P<0.001$).

Discussion

Demographic characteristics of the patients participated in the study showed that, the mean age was 51.7 years and above 60% were females. The authors attributed this finding to the combined effect of a greater number of elderly women than men and the increasing prevalence of diabetes with age. A significant difference in the prevalence of diabetes between males and females was documented in Saudi study[11]. Diabetes particularly

Table 5. Patients self- care activities correlated to age groups

Background characteristic	Age groups in years			Total	P value
	< 40	40-65	>65		
Regular exercise					
Yes	61 (41.2%)	167(42.0%)	37(29.8%)	265	0.049
No	87(58.8%)	231(58.0%)	87(70.2%)	405	
Regular doctor visit					
Yes	36(24.3%)	226(56.8%)	72(58.1%)	334	0.000
Sometimes	65(43.9%)	93(23.4%)	31(25.0%)	189	
No	47(31.8%)	79(19.8%)	21(16.9%)	147	
Diet plan at home					
Yes	43(29.1%)	158(39.7%)	54(43.5%)	255	0.001
Sometimes	46(31.1%)	151(37.9%)	43(34.7%)	240	
No	59(39.9%)	89(22.4%)	27(21.8%)	175	
Diet plan outside home					
Yes	11(7.4%)	98(24.6%)	37(29.8%)	146	0.000
Sometimes	57(38.5%)	145(36.4%)	46(37.1%)	248	
No	80(54.1%)	155(38.9%)	41(33.1%)	276	
Monitoring blood glucose level at home					
Yes	46(31.1%)	226(56.8%)	74(59.7%)	346	0.000
Sometimes	56(37.8%)	83(20.9%)	21(16.9%)	160	
No	46(31.1%)	89(22.4%)	29(23.4%)	164	

among urban–dwellers and older individuals was found to be prevalent among Omani population[12].

Out of all participants 61% were classified as knowledgeable about diabetes. Comparatively with a study in United Arab of Emirates by Al-Maskari *et al.*[13] found a lower percentage of the patients had poor knowledge about diabetes. The difference observed between the two studies in this domain may be explained by the fact that the Emirati study was conducted in university hospitals, where diabetes education may be more readily accessible to patients. However, in the current study some patients were recruited from primary health care facilities where diabetes education is less or not provided.

Significant association was documented between patients' total knowledge and their educational level. In contrary to this finding a recent study conducted among Qatari diabetic patients no association was noted between diabetes related– knowledge and patients educational level [14]. Higher education, no doubt, helps patients to understand the educational messages. In addition, such patients have better chances to come across a considerable knowledge about diabetes, it complications and treatment. Poor health literacy was found to be more common among elders and patients who have low educational attainment[15]. Inadequate health literacy was found to be an independent risk factor associated with both worse glycemic control and higher rate of diabetic visual complications[16]. Considering the fact that nearly one third of the participants were illiterates; future interventions in order to produce sound outcomes should be designed in a simplified manner to target these patients.

Despite the fact that the number of rural participants was small, but there was significant difference in their knowledge compared to the urban ones, who were found to be more knowledgeable. Variation in educational levels or dissimilarity in the provided health services may account for the observed difference in knowledge between the two groups.

Regarding patients' beliefs on predisposing factors of diabetes; considerable number of them correlate the onset of diabetes to the family history, obesity, stressful life, lack of physical activity and increased sugar intake. Actually, the correlation of all these factors and onset of diabetes were documented in the literature[17- 20], except sugar intake which was not found to play a deleterious role in primary prevention of type II diabetes[21].

The study revealed variations regarding participants' practices related to self-care activities: diet plan, exercise, and self glucose monitoring at home. Generally, the participants had bad practices. Poor practices towards diabetes among Saudi female patients were documented in a previous study[11].

Adherence to self-care measures was found to be significantly determined by respondents' age. Younger patients did exercise regularly compared to elder ones. Most of the elder participants were women and they most probably suffered from other coexisting illnesses, which may limit their activities. A study conducted to analyze the barriers to physical activity among elder diabetic patients; identified that, the most common barriers were: poor health, lack of company and lack of interest [22]. Self-efficacy and social support from family, friends, and health care providers were found to play an important role in adoption and maintenance of regular physical activity [23]. In contrary; elderly participants' practices related to adherence to dietary measures were found to be significantly better than younger ones. Younger being employed consume most of their daytime outside home so they may find difficulty in adhering to healthy diet. Many factors were identified in the literature that influence diabetic patients adherence to dietary measures such as the cost of healthy eating, small portion sizes, family support issues, and lifestyle issues, lack of knowledge and feeling of frustration from poor glycemic control despite adherence[24].

Interestingly no association was observed between participants' total knowledge on diabetes and their practices. The only exception was the percentage of patients with good knowledge was found to perform regular monitoring to their blood glucose level at home significantly more than patients with poor knowledge. In contrast other researchers found both knowledge and practices scores were low among diabetic patients[25].

Conclusion

The results of the current survey showed a gap in patients' knowledge on diabetes and its complications. In addition, patients practices related to self-care activities were sub-optimal.

Patients should be empowered through the provision of continuous educational program with special emphasis on illiterates, less educated and residents of rural areas. Continuous evaluation of patients' knowledge and practices will improve treatment outcomes.

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Psychological distress, coping style and immune activation in HIV-infected patients

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Abstract

Background: The progression of HIV infection is associated with significant levels of psychological distress. This study aimed to explore the correlation between perceived psychological distress, depression, coping style and immune activation in HIV-infected patients.

Materials and Methods: Participants included 110 HIV-infected patients from the Clinic of Infectious Diseases, Timisoara. Each patient completed a self-report encompassing perceived stress scale (PSS), Beck depression inventory (BDI) and the coping orientation to the problems experienced (Brief-COPE) scale. Immune activation was monitored by determining CD4 cell counts and viral load (VL).

Results: Among the study group, 26% had BDI scores for moderate depression and 3% for severe depression. Moreover, 24.5% of the patients had >10 scores on PSS. Higher levels of depression correlated with lower CD4 cells counts ($r = 0.275$, $p<0.004$) and with higher VL ($r = 0.211$, $p < 0.027$). Self-blaming negatively correlated with CD4, whereas behavioural disengagement correlated positively with VL.

Conclusion: Increased incidence of depression with higher levels of perceived psychological distress leads to maladaptive coping patterns associated with progression of HIV infection.

Key words: stress, coping, immune activation, HIV, depression

Introduction

Opportunistic infections and physical symptoms associated with HIV/AIDS, a negative perspective regarding the evolution of the disease as well as various forms of psycho-social stigma lead to increased levels of psychological distress and symptoms of depression in HIV-infected individuals. According to numerous studies, depression and impaired quality of life are associated with disease progression in these patients, who are confronted with long-term pain and suffering, dramatic changes in their lifestyle, prejudicial attitudes of others and social isolation (1,2,3). Retrospective statistical data indicates that the incidence of depression is up to two times higher in HIV patients than in the normal population (4,5).

The high incidence of depression can be explained through psychological and psycho-social mechanisms. As a result of the use of highly active antiretroviral therapy (HAART), HIV infection has become a rather chronic condition and not necessarily a life-threatening one. However, the infected people continue to experience seriously disturbing physical symptoms. The irrational fear of contamination and the association of AIDS with already marginalized groups (homosexuals, sex workers and drug addicts) have affected the social functioning domains of these patients. Four distinct forms of HIV-related stigma have been described; enacted, vicarious, felt normative and internalized, each of which lead to various degrees of intolerance, prejudice and discrimination, adverse attitudes directed towards the HIV-infected, disturbed self-concept, devalued social status, defensive reactions and social isolation (6).

Several studies revealed significant negative correlations between HIV-related symptoms of depression and CD4 cell counts (7,8,9). Some authors have suggested that the relation between psychological and immunological functioning in HIV infection is supported by the bidirectional interactions between the central nervous system and immune effector cells via sympathetic neurotransmitters, cytokines and hypothalamic–pituitary–adrenal hormones (10,11).

A review of research data about depression, psychological distress, coping strategies and immune function in AIDS/HIV-infected individuals indicates the existence of multiple and complex interactions between these variables. Following this line of research, the present study aims to explore the psychological status of a sample of HIV-infected individuals in a particular socio-demographical context, and to take one further step towards a model that integrates in a comprehensive framework, the psychological, social and biological dimensions of HIV/AIDS.

Material and methods

The study group included 110 participants (M=62; W = 48; mean age = 24.54 ± 12.35 years, 18–38 years) recruited from the Clinic of Infectious Diseases, Timisoara.

Psychological stress has been defined as a situation or an event that an individual assesses as exceeding her/his resources. Coping involves cognitive and behavioural strategies that people use consciously or unconsciously to reduce stress (12).

The perceived stress scale (PSS) was administered for assessing perceived psychological distress. This is a 14-item global scale designed to evaluate the degree to which the situations in one's life are appraised as stressful (14).

Depression was assessed with the classical Beck depression inventory (BDI-II; 15, whereas the coping orientation to the problems experienced (Brief-COPE) scale was used for investigating coping strategies used by the participants. COPE scale assesses the following coping strategies: self-distraction, active coping, denial, substance use, use of emotional support, use of instrumental support, behavioural disengagement, venting, positive reframing, planning, humour, acceptance, religion and self-blame (13). The markers VL and CD4 were selected as indicators of viral/disease progression and immune activation, respectively:

Results

Of the 110 patients studied, 25 were diagnosed with AIDS (CD4 ≤ 200 cells/mm^3), 45 were HIV-infected and received specific treatment (CD4 between 200–499 cells/mm^3) and 40 were diagnosed with HIV infection but did not follow any specific treatment (CD4 ≥ 500 cells/mm^3). The time elapsed since HIV diagnosis ranged between 12 years (diagnosed in 1998: 18 participants) and 1 year (diagnosed in 2010: three participants).

The socio-demographical data unfortunately illustrates the disadvantaged condition of these individuals: their education level was extremely low [42 (38%) participants graduated from only primary school and 68 (62%) graduated from secondary school]; 15 (14%) participants were married and 95 (86%) were unmarried; 42 (38%) were residents in urban areas and 68 (62%) in rural areas; only 14 (13%) participants had a permanent job and 96 (87%) were unemployed.

BDI-II is a psychometric instrument widely used for assessing clinical depression. BDI contains 21 questions about how the subject has been feeling for the last 2 weeks, each answer being scored from 0 to 3. Higher total scores indicate more severe depression symptoms. According to the BDI-II manual (15), scores between 0–4 indicate the absence of depression; scores of 5–13 signify minimal depression; 14–19, mild depression; 20–28, moderate depression and 29–63, severe depression (Table 1). Mean score for the group was 16.15 and the total score range: [5–29].

Pearson correlation analysis indicated a significant correlation between the time elapsed since HIV diagnosis and depression ($r = 0.235$, $p < 0.013$).

PSS contains 14 questions, each scored from 0 to 4 points. The total score is obtained by summarizing the scores of all questions, with half of them being reverse coded. The possible values fall in the range [−24, +24].

Table 1. Distribution of BDI-II scores in the study group

BDI Score range	Number of cases	Percent (%)	Interpretation
0–4	0	-	No depression
5–13	35	31.81%	Minimal depression
14–19	43	39.09%	Mild depression
20–28	29	26.36%	Moderate depression
29–63	3	2.73%	Severe depression

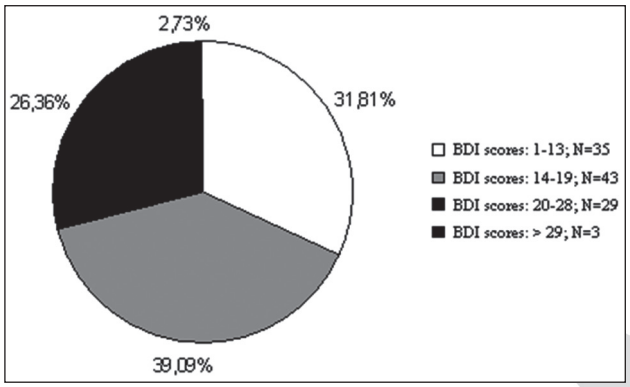

Figure 1. Distribution of depression levels in the study group

Higher positive scores indicate higher perceived psychological distress. Negative values indicating low perceived psychological distress were obtained for N = 22 (20%) patients, whereas positive values indicating high perceived psychological distress were obtained for N = 88 (80%) patients; 27 (24.5%) of these cases had extremely high scores (>10 points). Mean score of the group was 5.34 and total score range [-12, 15].

Brief-COPE scale has been administrated to identify the strategies that AIDS/HIV-infected individuals use most frequently to cope with their emotional and psycho-social problems. In our sample of HIV-infected individuals, the COPE scale indicated the following results (Table 2).

The immunologic markers had the following distribution (Table 3).

Table 2. Coping strategies: mean scores in the study group

Coping strategies	Mean Score (n=110)
Self-distraction	5.11* (SD = 1.31)* *
Active coping	5.17 (SD = 1.17)
Denial	4.36 (SD = 1.28)
Substance use	1.92 (SD = 1.13)
Use of emotional support	5.24 (SD = 0.93)
Use of instrumental support	5.07 (SD = 0.99)
Behavioral disengagement	4.19 (SD = 1.20)
Venting	5.75 (SD = 1.01)
Positive reframing	4.46 (SD = 1.25)
Planning	4.20 (SD = 1.75)
Humour	2.64 (SD = 1.63)
Acceptance	5.89 (SD = 1.05)
Religion	6.54 (SD = 1.06)
Self-blame	1.95 (SD = 1.48)

** For each subscale, the minimum possible score is 0 points and the maximum 8 points.*

*** The values in brackets represent standard deviation.*

Table 3. Distribution of immunologic markers in the study group

Distribution of the CD4 blood counts	
CD4	**Number of cases**
≥500 cells/mm^3	40
200-499 cells/mm^3	45
≤200 cells/mm^3	25
Viral load distribution	
VL	**Number of cases**
undetected	30
1000-10000 copies/ml	35
10000-50000 copies/ml	25
>50000 copies/ml	20

Year of diagnosis	Number of cases
1998	18
1999	20
2000	15
2001	7
2002	8
2003	7
2004	5
2005	6
2006	5
2007	7
2008	5
2009	4
2010	3

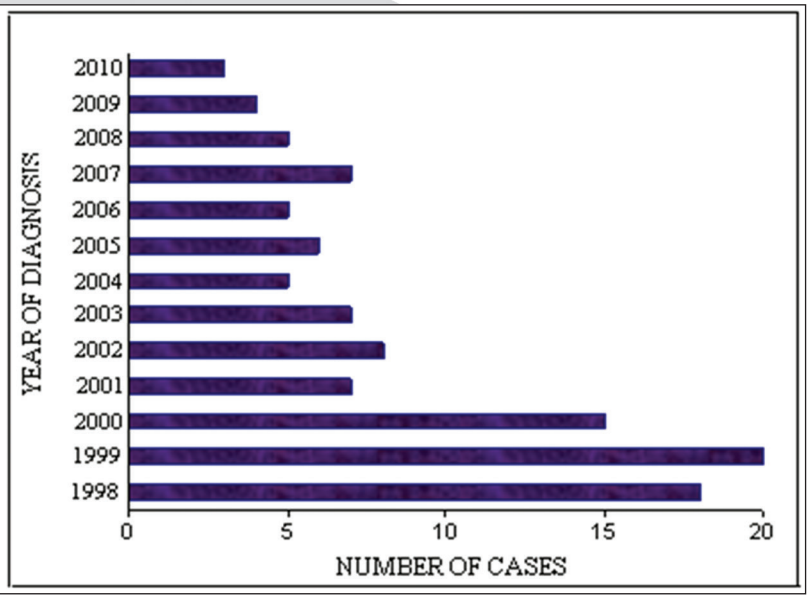

Figure 2. Distribution of the time elapsed since HIV diagnosis in the study group

Multiple correlations between coping style, depression, perceived psychological distress and immunologic markers led to the following values (Table 4).

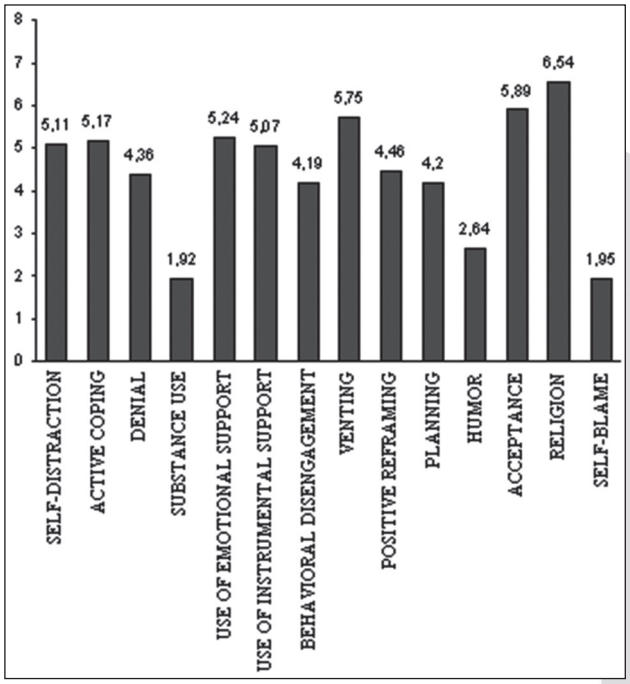

Figure 3. Coping strategies: distribution of the mean scores in the study group

Discussion

The distribution of data reveals that AIDS/HIV infection significantly impairs the quality of life, leading to significant rates of depression and perceived psychological distress. In our study group, 26% of the participants had BDI scores that qualify them as having moderate depression, and 3%, as having severe depression. Regarding the perceived psychological distress, 24.5% of the participants had very high scores (>10 points on PSS).

The high rates of depression and perceived distress can be explained as psychological reactions to multiple stressors associated with HIV infection: changes in lifestyle, perceived social stigma, social isolation, behavioural restrictions and impaired self-concept resulting from enacted, vicarious and internalized forms of stigma, physical symptoms such as pain or fatigue and a high incidence of opportunistic infections.

More recently, several studies have identified a common biochemical pathway that explains the correlation between HIV/AIDS progression and clinical depression (16,3). The fact that the decreased availability of both serotonin and tryptophan

Table 4. Multiple correlations between coping style, depression, perceived psychological distress and immunologic markers

	Depression	Perceived psychological distress	CD4	VL
Self-distraction	r=0.137 p<0.152	r= −0.084 p<0.383	r=0.133 p<0.079	r=0.130 p<0.167
Active coping	r=−0.339** p<0.001	r = −0.201* p<0.035	r=0.216* p<0.023	r= −0.219* p<0.022
Denial	r=0.187 p<0.052	r = −0.212* p<0.026	r = 0.056 p<0.540	r=0.173 p<0.070
Substance use	r=−0.016 p<0.870	r=0.216* p<0.023	r=0.181 p<0.059	r=0.005 p<0.962
Emotional support	r= −0.099 p<0.301	r = −0.175 p<0.068	r=0.078 p<0.415	r=−0.186 p<0.052
Instrumental support	r=−0.223* p<0.019	r=−0.217* p<0.023	r = 0.166 p<0.074	r=−0.093 p<0.335
Behavioural disengagement	r=0.061 p<0.524	r=−0.212* p<0.026	r=−0.225* p<0.018	r=−0.021 p<0.894
Venting	r=0.169 p<0.078	r=0.047 p<0.626	r=−0.021 p<0.824	r=0.043 p<0.653
Positive reframing	r=−0.324** p<0.001	r= −0.223 p<0.019	r=0.307** p<0.001	r=−0.240* p<0.011
Planning	r=0.066 p<0.494	r=−0.080 p<0.407	r=0.186 p<0.052	r=−0.025 p<0.792
Humour	r=−0.179 p<0.061	r=−0.101 p<0.254	r=0.079 p<0.410	r=0.030 p<0.753
Acceptance	r=0.168 p<0.079	r=−0.211* p<0.027	r=0.179 p<0.062	r=−0.064 p<0.506
Religion	r=−0.204* p<0.032	r=−0.180 p<0.061	r=0.195* p<0.041	r=−0.155 p<0.106
Self-blame	r=0.224* p<0.020	r=−0.155 p<0.106	r=0.091 p<0.346	r=0.224* p<0.019
Depression	1	r=0.664** p<0.001	r=−0.275** p<0.004	r=0.211* p<0.027
Perceived psychological stress	r=0.664* p<0.001	1	r=−0.195* p<0.041	r=0.168 p<0.079

** p < 0.05, ** p < 0.01*

(an essential amino acid precursor of serotonin) has an important role in the pathogenesis of mood disturbances and depression is well documented in psychiatric literature. In HIV infection, enhanced tryptophan degradation has been observed, this process being actively inhibited by HAART. Thus, it has been hypothesized that tryptophan degradation may be causally linked to the increased rate of depression in HIV-infected individuals.

At the psychological level, a positive diagnosis may lead to dysfunctional beliefs and negative emotions (mental rumination, permanent worries, exaggerated self-blame, anxiety, anger or sadness), with these people having to cope not only with a severe and stigmatizing illness, but also with life stressors such as goal obstructions, losses, family crises or even abandonment from loved ones. Thus, the high levels of perceived distress and depression in HIV-infected individuals are viewed by some authors as consequences/reactions to their physical, psychological and social problems (17,18).

The poor educational status of the HIV-infected individuals in Romania could be explained by the fact that most of them contracted the virus from birth or in early childhood; in the latter case, it was usually by inadequately performed medical treatments in foster care institutions. Furthermore, most of them quit school and isolated themselves, being victims of stigmatization and social rejection.

Hierarchically, the coping strategies most frequently employed in HIV infection were the following: religion (mean = 6.54), acceptance (mean = 5.89), venting (mean = 5.75), active coping (mean = 5.17), self-distraction (mean = 5.11) and use of instrumental support (mean = 5.07). These coping preferences also reflect the cultural characteristics of the people in the study group (the socio-demographic data indicate some particularities of the people included in the study, namely, that most of them live in rural areas and have a low educational and professional status). The coping strategies that people most frequently use seem to have a positive emotional and even immunologic impact. For instance, the use of religion correlated with lower symptoms of depression ($r = 0.204$, $p < 0.032$) and with higher CD4 ($r = 0.195$, $p < 0.041$), whereas acceptance significantly correlated with lower perceived psychological distress ($r = 0.211$, $p < 0.027$). Active coping seems to be the most efficient strategy, which significantly correlated with lower depression ($r = 0.339$, $p < 0.001$), reduced symptoms of perceived psychological distress ($r = 0.201$, $p < 0.035$), higher CD4 ($r = 0.216$, $p < 0.023$) and lower VL ($r = 0.219$, $p < 0.022$). Previous studies have also indicated the efficacy of spirituality in improving well-being in HIV-infected individuals and its positive impact on immune activation in these patients (19).

Multiple correlation analysis performed between the coping strategies that people use, depression, perceived psychological distress and immunologic markers indicates that the most appropriate coping strategies are: active coping, positive reframing, instrumental support and religion. Self-blaming negatively correlates with CD4, and behavioural disengagement correlates positively with VL. These data suggest that such strategies might have a negative impact on immune activation, at least in HIV infection. Other studies also indicate that active instrumental and problem-focused coping strategies have a positive impact on immune activation in AIDS, with disengagement and avoidant coping being regarded as maladaptive at this level (20). In our sample of HIV-infected individuals, substance use (mean = 1.92), self-blame (mean = 1.95) and humour (mean = 2.64) are the least used coping strategies for dealing with HIV infection.

Depression has been the variable that correlated most significantly with immune markers. Higher levels of depression correlated with lower CD4 ($r = 0.275$, $p < 0.004$) and with higher VL ($r = 0.211$, $p < 0.027$). In a regression analysis, depression explained 26% of the variance in CD4 ($F = 0.921$, $p < 0.027$). Our data indicate that living with HIV infection makes people more vulnerable to depression, as the depression rates positively correlated with the time elapsed since the patient became aware of the positive diagnosis. The result indicates that more psychological support should be offered to these patients.

Conclusions

Our results indicated a significant incidence of depression and a high incidence of perceived psychological distress among HIV-infected patients. Depression is the most important predictor of immune status. Higher levels of perceived psychological distress and maladaptive coping patterns also significantly correlated with disease progression.

These data confirm a psycho-immune model of HIV progression, and highlight the importance of considering the psychological variables in order to optimize the long-term interventions in HIV-infected patients.

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71 cases Klebsiella pneumoniae bloodstream infections in Preterm infants

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Abstract

Objective: To study the clinical characteristics of Klebsiella pneumoniae bloodstream infection in preterm infants.

Methods: The clinical data of premature infant bloodstream infection with Klebsiella pneumonia in 71 cases that were divided into early-onset group and late-onset group were studied from 6 February, 2008 to 10 February, 2010 in NICU wards. The perinatal data, clinical manifestations, laboratory examination that Klebsiella pneumoniae infection occurred including: complete blood count, CRP, liver and kindey functions were analyzed, using two independent samples t-test or t'-test, Spearman correlation analysis and non-conditional Logistic regression analysis for research.

Results: 71 of total 2721 admitted premature infants infected with Klebsiella pneumoniae by bloodstream, infection rate 2.61%. There were early - onset infections in 63 cases, late-onset infections in 8 cases. Clinically, Klebsiella pneumoniae bloodstream infection were characterized by poor response, quiet state of the heart rate > 160 beats / min, oxygen saturation decreased. Skin color changes, apnea and groaning accounted for over 80%, followed by jaundice, and changes in body temperature. I / T ratio and CRP were not statistically significant between early-onset group and late-onset group when Klebsiella pneumoniae bloodstream infection occurred. Early-onset Klebsiella pneumoniae sepsis were associated with CRP (OR=1.016, 95%CI: 1.031-1.575, p=0.0042), I/T (OR=1.282, 95%CI: 1.024-1.467, p=0.048) and birth weight (OR=1.623, 95%CI: 1.121-2.304, p=0.039). The recovery rate had a negative correlation with premature rupture of membranes time and hospital length of stay, r=-0.450, -0.468, p=0.006, 0.001; Drug susceptibility showed high antibiotic resistance to penicillin and two or three-generation cephalosporins, but carbapenems and quinolones were susceptible.

Conclusions: The clinical manifestations of Klebsiella pneumoniae sepsis in preterm infants are non-specific. It is necessary to maintain vigilance for Klebsiella pneumoniae infection in preterm infants with non-specific infection clinical manifestations, low birth weight, invasive operation, I / T and CRP levels increases.

Key words: prematurity; Klebsiella pneumoniae; neonatal septicemia; CRP;

Introduction

Klebsiella pneumoniae (K. Pneumonia) was a facultative anaerobic gram-negative bacilli, no flagella, with a thick capsule and the majority of strains owning pili. It was the most common bacteria of human respiratory tract and intestinal tract and also an important opportunistic pathogen. Infants are predisposed to K. Pneumonia, especially in premature infants with immune dysfunction and can cause a wide variety of infections including respiratory tract infections, urinary tract infections, intestinal tract infections, bloodstream infections (BSIs), operative incision infections, skin and soft tissue infections, other parts of the body infections [1]. In recent years, with the success rate of resuscitation at birth improved in premature infants, especially in very low birth weight infants, K. pneumoniae infection has become primary risk factors for the premature infant survival in many neonatal wards. Columbia Neonatal Research Network in South America, a prospective study on eight wards has found that bloodstream infection was the most common neonatal infection route, accounting for 10.0 ~25.0%, 50.0% of the total very low birth weight infants[2]. Preterm infants onset was insidious, rapid development, extremely dangerous[3]. Clinical manifestations were non-specific, atypical and often overshadowed by the primary disease of preterm infants, and easily combined with other bacterial or fungal

infections. Blood culture was currently the gold standard for the diagnosis of sepsis. However, the fact that culture reports were available only after 48-72 hours could not promptly offer guidelines for treatment, resulting in pathogenetic condition irreversible. How to base on early clinical features and to judge correctly the K. pneumoniae infection was challenging faced by neonatology doctor. This article analyzed of 71 cases K. pneumoniae bloodstream infection in premature infants. The reports were as follows.

This article was research on clinical characteristics of K. pneumoniae in preterm infant septicemia. Procedures were performed according to the Helsinki Declaration and approved by institutional Review Board (Institutional Review Board of The General Military Hospital of Beijing PLA). The blood samples of preterm infants were collected according to preterm infant condition after approval was obtained from their families during hospitalization.

Materials and Methods

The clinical conditions of preterm infants

A total of 2721 cases preterm infants were admitted to preterm infants intensive care unit (with 120 beds) of BaYi Children's Hospital from 6 February, 2008 to 10 February, 2010. Of the 2721 admitted preterm infants, 71 (2.61%) were infected K. pneumonia. Of the 71 preterm infants K. pneumonia infection, Sixty-six point two percent were male. 3 cases were vitro fertilization - embryo transfer babies. Gestational age (GA) ranged from 25 weeks to 36 weeks and the mean GA was 31.296 ± 2.759 weeks. Very premature birth <28 weeks in 7 cases, 28-31 weeks in 31 cases, 32-36 week in 33 cases. Delivery methods: there was spontaneous delivery in 31 cases (43.7%), uterine-incision delivery in 40 cases (56.3%). Birth weight (BW) ranged from 600g to 3150g and the mean birth weight was 1598.3 ± 543.504g. Hospitalzation day ranged from 7 days to 128 days and an average of hospitalzation day was 46.732±27.372 days.

The clinical conditions of mothers of 71 preterm infants

There was fetal distress in 35 cases. Hemorrhagic amniotic fluid was 3 cases. One, two and three degree amniotic fluid contamination were 10, 26, 11 cases, respectively. Antenatal steroids were administrated in 16 cases. Mother was suffering from chorioamnionitis in 3 cases, pregnancy-induced hypertension and severe pre-eclampsia in 6 cases, gestational diabetes in 12 cases. Partus precipitatus and velamentous placenta were each 2 cases. Premature rupture of membranes(PROM) were 71 cases and ranged in time from 0.5h to 384h, less than 24 hours in 12 cases,24 ~ 48 hour in 22 cases, 48 ~ 72 hours in 28 cases, more than 72 hours in 9 cases.

Diagnosis criteria

The diagnostic criteria of K. pneumoniae sepsis: the two blood cultures results were concordance at the same time in different parts of the body.

Underlying disease

Diagnosis criteria for underlying diseases consulted Practice of Neonatology fourth Edition. The most common underlying illness was apnea in 60 cases, neonatal hyperbilirubinemia in 60 cases, followed by type I respiratory failure in 52 cases, neonatal respiratory distress syndrome(NRDS) in 38 cases, scleredema neonatorum in 31 cases, neonatal pneumonia in 27 cases, hypoglycaemia in 19 cases, intracranial hemorrhage in 8 cases, neonatal asphyxia in 8 cases, preterm brain changes in 8 cases, septic shock in 7 cases, multiple system organ failure in 6 cases, type II respiratory failure in 6 cases, pulmonary hemorrhage in 5 cases, hypoxic-ischemic encephalopathy (HIE) in 5 cases, disseminated intravascular coagulation(DIC) in 4 cases, hydrocephalus in 3 cases, renal failure in 3 cases, congenital cataract in 3 cases, biliary atresia in 3 cases ,righ lung atelectasis in 2 cases, patent ductus arteriosus in 2 cases, twin-to-twin transfusion syndrome in 2 cases, thyroid dysfunction in 1 case, atrial septal defect in 1 case.

Laboratory examinations

Blood samples collected: Blood cultures were drawn in different two parts of the body when preterm infants were suspected sepsis according to the clinical manifestations. At the same time blood samples for complete blood count, CRP, kindey and liver functions were drawn. Antibiotics have been used before blood sample were obtained. Sputum cultures were done using endotracheal suction via endotracheal intubation.

Semi-automatic immune-turbidimetry device TURBOXPLUS from ORION Company, CRP Assay Kit were purchased from ORION Company. COBAS INTEGRA 400 plus automatic biochemical Analyzer was manufactured by Roche Company, Germany.

Susceptibility test

VITEK-32 automatic bacterial analysis system was used. GNS-120 drug susceptibility analysis cards and sensitivity agar (MH) were the France BioMerieux products.

Research methods

These cases diagnosed as K. pneumoniae infection were obtained from 2721 admitted premature infants. Clinical manifestations, laboratory examinations, blood culture, sputum culture and drug sensitivity test results were recorded. Related perinatal factors of K. pneumoniae infection and the change law of inflammatory indicators occurred K. pneumoniae infection were analyzed.

Statistical analysis

All date were analyzed using the SPSS16.0 software, two independent sample t or t' test for measurement data, bivariate correlation analysis Spearman's correlation coefficient for correlation analysis and non-conditional Logistic regression analysis for these variables were performed. P-value of 0.05 or lower was considered statistically significant.

Results

According to the literature[4], K. pneumoniae sepsis was divided into early- onset sepsis (EOS, onset age ≤72h) in 63 cases and late-onset sepsis (LOS, hospital- acquired infections, onset age> 72h) in 8 cases.

Clinical manifestations

Clinically, K. pneumonia infections in preterm infants were characterized by poor response in 71 cases (100%), the quiet state of heart rate over 160 beats / min in 71 cases (100%), blood oxygen saturation decrease in 71 cases (100%), marble-like skin color change in 65 cases (91.5%), gray skin color in 59 cases (83.1%), groaning in 58 cases (81.6%), apnea in 58 cases (81.6%), facial complexion cyanosis in 55 cases (77.5%), abdominal distention in 48 cases (67.6%), fever in 40 cases (56.3%), gastric residual milk increase in 39 cases (54.9%),more viscous Sputum in 36 cases (50.7%), jaundice aggravation in 32 cases (45.1%), hypothermia in 25 cases (35.2%), persistent jaundice in 23 cases (32.4%), normal body temperature in 10 cases (14.1%), hepatosplenomegaly in 32 cases (45.1%), auscultation lung rales in 58 cases (81.7 %),accompanied with septic shock in 7 cases and DIC in 4 cases.

The clinical features of nosocomial infection in 8 cases were: mechanical ventilation in 6 cases, CPAP in 1 case, PICC in 1 case. The body temperature was normal when infections occurred. Mainly blood oxygen saturation could not be maintained by assisted ventilation and needed to increase ventilator parameters, followed by apnea, more viscous sputum. NO brick-red gelatinous-like sputum was found. The color of skin was dark gray and jaundice faded delay.

Pathogenic bacteria culture

K. pneumoniae sepsis were 71 cases including early-onset sepsis in 63 cases, accounting for 88.7% (63/71) and 2.32% (63/2721) of the same period hospitalized premature infants. In synchronous blood culture, there were Candida tropicalis in 3 cases and Candida krusei in 1 case. Group A streptococcus in 6 cases and Candida albicans in 2 cases were found in sputum culture. Hospital acquired K. pneumoniae sepsis were 8 cases, accounting for 11.3% (8/71) of K. pneumoniae sepsis and 0.29% (8/2721) of the same period hospitalized preterm infants. There were oral streptococci in 2 cases and brevendimonas diminuta in 1 case in synchronous blood culture.

Cerebrospinal fluid culture

Cerebrospinal fluid culture in 63cases, no K. pneumoniae was found.

Chest X-ray

Chest X-ray showed that the principal performance of lung were spot-like, patchy shadow, superior lobe of right lung atelectasis in two cases.

Compared with complete blood count and CRP between the two groups when K. pneumoniae infection occurred

Complete blood count and CRP were tested when the blood culture were drawn in 71 cases preterm infants infected with K. pneumoniae. These results showed that the values of WBC, N, Plat, I / T, HB, MCV, MCH, CRP were no difference between early-onset sepsis group and late-onset sepsis group, t values were: 0.596, 0.131, 0.227, 0.125, -0.195, -1.224, -1.262, 0.171, respectively.

Logistic regression analysis about the related factors for 63 cases early - onset K. pneumoniae sepsis

Analysis 63 cases early-onset K. pneumonia sepsis associated with the each indicator of complete blood count, liver and kidney function that these blood samples were simultaneously obtained in blood culture and perinatal factors including gender, GA, delivery methods, fetal distress, amniotic fluid contamination, BW, antenatal steroids, hospital stay and PROM in the 71 cases K. pneumoniae sepsis. Spearman's correlation analysis results showed that MCV, MCH, CRP, DBIL, BUN, ALB, I/T (Table1), delivery methods and birth weight (Table 2) were statistical significance, but Non-conditional Logistic regression analysis results showed that only CRP, I / T and birth weight were correlated with early-onset K. pneumonia sepsis (Table 3).

The resistance rates of 71 strains of K. pneumoniae infection

The data shows that resistance of K. pneumoniae emerges mostly to penicillin, second and third generation cephalosporins, but there is no case of the emergence of resistance to quinolones and carbapenems (Table 4).

Treatments strategies

39 (54.9%) of the 71 cases were treated with Ventilator, 21 (29.6%) cases with CPAP, 29 (40.8%) cases with surfactant. The appropriate sensitive antibiotic were chosen for treatment 2 to 3 weeks based on drug susceptibility, then blood and sputum culture were re-examined until it turned to negative. Conventional therapy included keeping body warm, nasal feeding, parenteral nutrition, phototherapy, treatment jaundice drugs, plasma, albumin, correct acidosis, heparin and other symptomatic and supportive treatment, complicated fungal infection with antifungal treatment.

Table 1. The Spearman's correlation analysis between early-onset K, pneumonia sepsis and each examination marker

	MCV	MCH	CRP	DBIL	BUN	ALB	WBC	N	PLat	TBIL	I/T	RBC	HB
r	0.444	0.351	-0.668	0.429	0.809	0.701	-0.195	-0.216	0.211	0.221	0.649	0.261	0.237
p	0.004*	0.026*	0.000*	0.006*	0.000*	0.001*	0.228	0.181	0.192	0.171	0.002*	0.103	0.142

Table 2. The Spearman's correlation analysis between early-onset K, pneumonia sepsis and perinatal factors

	gender	GA	delivery methods	fetal distress	amniotic fluid contamination	BW	antenatal steroids	hospital stay	PROM
r	0.141	0.158	-0.240	0.086	0.053	0.350	-0.049	-0.158	-0.115
p	0.229	0.175	0.038*	0.597	0.744	0.031*	0.765	0.176	0.481

**p<0.05*

Table 3. Logistic regression analysis about the related factors for early - onset Klebsiella pneumoniae sepsis in 63 cases

Factor	B	SE	Wald	P	OR	95% CI for Exp(B)	
						Lower	Upper
BW	0.016	0.009	6.258	0.042	1.016	1.031	1.575
I/T	0.249	0.139	5.215	0.048	1.282	1.024	1.467
CRP	0.327	0.235	4.927	0.039	1.623	1.121	2.304

Table 4. The resistance rates of 71 strains of K. pneumoniae infection

Aantibiotics	Sensitive strains	Intermediary	Resistance strains	Resistance rates (%)
Ampicillin	0	2	69	97.2
Ticarcillin	2	4	65	91.5
Amoxicillin/Sulbactam	47	8	16	22.5
Cephazolin	0	0	71	100.0
Ceftriaxone	0	5	66	92.9
Cefotaxime	0	3	68	95.8
Cefoperazone	0	4	67	94.4
Ceftazidime	3	6	62	87.3
Cefepime	4	15	52	73.2
Ciprofloxacin	71	0	0	0.0
Levofloxacin	71	0	0	0.0
Amikacin	61	8	2	2.8
Imipenem	71	0	0	0.0
Meropenem	71	0	0	0.0
Aztreonam	8	11	52	73.2

Table 5. Spearman's correlation analysis about perinatal factors for influence Prognosis:

	gender	GA	delivery methods	fetal distress	amniotic fluid pollution	BW	antenatal steroids	hospital stay	PROM
r	0.090	0.046	0.109	0.226	-0.146	-0.116	0.034	-0.468	-0.450
p	0.457	0.706	0.365	0.186	0.395	0.334	0.845	0.002*	0.006*

**p<0.05*

Prognosis and influencing factors

There were 64(90.1%) preterm infants cured and 7 (9.9%) preterm infants died in 71 cases preterm infants K. pneumoniae sepsis. Perinatal factors for influence recovery rate were negatively correlated with the duration of premature rupture of membranes and hospital stay (Table 5). The duration of premature rupture of membranes of death in 7 preterm infants was more than 72 hours in this group.

Discussion

Zaidi[5] studies have shown that pathogens of early bacterial infection (early onset neonatal infection, EONI) within the first week of life (3209 isolates), K. pneumoniae species (25%), Escherichia coli (15%), and Staphylococcus aureus (18%) were major pathogens. Group B streptococci (GBS) were relatively uncommon (7%). The major pathogens of ENOI were dwelling flora of vagina and mostly by vertical transmission. Our hospital (The General Military Hospital of Beijing PLA -Affiliated Tertiary-Care Children's Hospital) was the Transit Center of Newborn Critical Care in Beijing and even in North China Area. Neonatal intensive care unit (NICU) concerned is 350 beds including full-term neonatal ICU ward and preterm infant ICU ward. More than 95% of critical newborn hospitalized were transferred from the other hospitals. All of pregnant mother in this group had premature rupture of membranes and the longest time of PROM was 384 hours, more than 72 hours in 9 cases, 48 h ~ 72 h in 28 cases. The incidence of PROM was 19.53% to 25%[6,7], About 30% to 40% of PROM was preterm premature rupture of membranes (PPROM) [8], more than 50% of PROM associated with chorioamnionitis and intra amniotic cavity infection [9].As a natural barrier, Embryonic membrane has a good protective effect for fetus. If fetal membranes have been broken, its protective effect lost, intra-amniotic cavity infection caused by vaginal bacteria direct into the amniotic cavity would undergo. At the same time, the vaginal environment has changed from the weak acid turning to weak alkaline due to PROM, so there is a big advantage for microbial growth. Infection was considered to be an important factor leading to PROM. Infection and PROM interacted with each other. Related

pathogens were mostly intravaginal conditions bacteria existing in cervix and fundus of vagina, such as Group B Streptococcal, Gonococci, Bacteroides, Chlamydia trachomatis, ureaplasma urealyticum and so on. All of these bacteria were associated with PROM [10]. Infection was often acquired within the first 72 hours after initial rupture of fetal membranes in PPROM. The incidence of preterm infant sepsis was still 5-8% even if prophylactic antibiotics treatment has reduced the neonatal sepsis in PPROM [11]. K. pneumoniae was no-intravaginal colonization bacteria, the possible sources of K. pneumoniae in this group required further study.

Spearman's correlation analysis shows that delivery methods and BW were significant (Table 2).However, Logistic regression analysis show that only BW was into regression equation. Cesarean section accounted for 56.3% in this group. Cesarean section itself was not a high risk factor caused early-onset sepsis of K. pneumonia. Cesarean section was selected because of poor fetal conditions caused by intrauterine infection. Newborn was almost impossible to touch the mother's flora during the process of cesarean section, replacing it with bacteria in hospital environments. Cesarean section and EOS may be the result of intrauterine infection owing to PPROM. Only birth weight was associated with K. pneumoniae infection in early-onset infection. Birth weight was less than 1500g in 26 cases (36.7%) in this group. The lower birth weight, the higher infection incidence[12]. Compared with full-term newborns, immune system development in preterm infants was poor. Although immunoglobulin IgG can pass through the placenta, but the smaller gestational age, the lower birth weight, the lower levels IgG through the placenta to reach the body.

This study showed that all cases clinical manifestations were characterized by poor response, the quiet state heart rate over 160 beats / min and blood oxygen saturation decreased. The incidence of marble-like skin color change, gray skin color and groaning were more than 80%, followed by jaundice and body temperature changes. The early-onset infection manifestations mainly were body temperature change, apnea and jaundice. Late-onset infection major manifestations were that blood oxygen saturation could not be maintained by assisted ventilation if not up-regulated ventilator parameters, followed by breathing rhythmicity change, more viscous sputum, feeding intolerance, jaundice relapse. All of these were non-specificity, so it was difficult to early definite diagnosis. All of the preterm infants of late-onset infection had clearly invasive operation. Preterm infants, in particular extremely low birth weight premature infants, various aggressive operations such as mechanical ventilation, aspiration of sputum, artery and vein puncture intubation and parenteral nutrition were inevitable. It was very easy to cause K.pneumoniae infection due to immunocompromice, poor resistance, weak defensive function in addition to all manner of invasive operation in preterm infant [13], but the pathogenic inducement were preventable. Therefore, health care workers must strictly obey medical procedures to reduce the incidence of infection. Literature reported that meningitis and brain abscess caused by K. pneumoniae sepsis lasted for a long time and the clinical manifestations were not typical and easy to occur in patients with diabetic[14] and liver transplantation surgery[15-16], and was related to K. pneumoniae genotype[17]. Cerebrospinal fluid culture in 65 cases confirmed that no case complicated central nervous system infection in this group due to treatment timely.

The laboratory tests showed that complete blood count and CRP were no differences between early-onset infection and late-onset infection group. It was consistent with the literature[18]. Spearman's correlation analysis show that Seven indicators (MCV, MCH, CRP, DBIL, BUN, ALB,I/T) were significant when K. pneumonia sepsis occurred (Table 1). However, Logistic regression analysis show that only I/T and CRP were associated with K. pneumoniae infection, indicating that I / T and CRP were sensitive indicators of K. pneumoniae infection. CRP had a certain value not only for early diagnosis[19], but also for guiding treatment .Ehl S[20] thought that: as far as newborn of clinical diagnosis of EONI was concerned, antibiotic treatment stopped as long as clinical manifestations of infection disappeared and plasma CRP levels were less than l0mg/L.

Third-generation cephalosporin with a broad spectrum antibacterial activity, low toxicity and resistance enzymes (non-ESBL), was one of the most commonly used antibiotics in NICU. Under

the selection pressure for usage these agents, it easily induced Enterobacter, especially K. pneumonia and Escherichia coli to produce extended-spectrum β-Lactamases (ESBLs)[21], thus led to one or more of the methoxy-imino β-lactam antibiotics resistance. The rate of K. pneumoniae producing ESBLs was higher from 10% to 40%[22]. Once formed, the resistance of ESBLs-producing bacteria can transfer to other gram-negative bacteria via plasmid-mediated, causing drug resistance diffusion[23]. All these characteristics can easily cause an outbreak of nosocomial infections. ESBLs-producing K. pneumoniae can attach to the surface of the body's mucous membranes and biological materials to form a biofilm which protect the bacterium to evade host immune and not to be killed by antibacterial agents, producing resistance to various antibiotics. Literature reported that low birth weight infant and early usage third-generation cephalosporins were major risk factors for ESBLs-producing bacterium infections [24]. Moodley and other studies had found that glucose infusion was one of these factors causing ESBLs strains spread proliferation[25]. Neonatal mortality of ESBLs-producing K. pneumoniae septicemia was 57% in NICU[21] and Shanmuganathan reported the mortality was 80%[26].The mortality of preterm infants in this group was 9.3% due to be dealt promptly. Most of premature infants have been used two or three generation cephalosporin before admitted, so these data (table 4) show that K. pneumoniae to penicillin, second or third-generation cephalosporins and aztreonam have very high resistance. In an attempt to reduce the prevalence of ESBL producers, we restricted the use of extended-spectrum cephalosporins and encouraged the use of β-lactam/β-lactamase inhibitor combinations (piperacillin/tazobactam or ampicillin/sulbactam) for empirical therapy and/or specific therapy. Fluoroquinolones and carbapenem antibiotic such as imipenem and meropenem are sensitive drugs. Fluoroquinolones should be caution application in preterm infants. Imipenem with a strong bactericidal effect is not recommended for bacterial meningitis due to not through the blood - brain barrier, but meropenem can be applied to meningitis.

In this study, the duration of premature rupture of membranes and hospital stay were associated with the recovery rate (Table 5). The longer the duration of premature rupture of membranes and hospital stay, the worse the recovery rate. One-third of prematures delivery caused by PPROM. PPROM accounted for a significant part of overall perinatal mortality and morbidity[27]. Amniotic fluid leaked continuously after PROM resulting in amniotic fluid decreased, umbilical cord Compression, following fetal hypoxia, fetal distress, neonatal asphyxia, pulmonary hypoplasia and restrictive deformity, even neonatal death[28]. Long-term effects of infection caused by PROM led to cytokine levels of umbilical cord blood increase and placental tissue degeneration. These would induce fetal brain injury, periventricular leukomalacia, bronchopulmonary dysplasia, pulmonary arterial hypertension. Amniotic fluid loss resulted in the high incidence of neonatal asphyxia and necrotizing enterocolitis. Perinatal mortality and bronchopulmonary dysplasia were closely related to the occurrence of time and persistent period of PPROM as well as the degree of amniotic fluid reduce[29]. Generally, the longer PPROM, the smaller gestational age, the more complications, the higher mortality rate.

This study has several limitations. Since most of preterm infants were transferred from other hospitals, failed to thoroughly record the data of mother during pregnancy, particularly whether infection occurred after PROM, how to select antibiotic and the duration of application antibiotic, placental pathology, blood culture, etc, as well as the information including the specific health care operations before the preterm infants were transferred and NICU infection situation from transport hospital failed to obtain. Possible sources of K. pneumonia infection can not make a detailed study which also requires a multi-center study.

Notwithstanding its limitation,this study does suggest that it is necessary to be cautious K. pneumoniae infection and should be prompt and proper treatment to a preterm infant with low birth weight, prolonged PROM time and no-specificity clinic manifestations , invasive operation, I / T and CRP increase, especially, blood oxygen saturation cannot be maintained when mechanical ventilation is going.

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Effect of four weeks of resistance training on liver glycogen and lipid profile in diabetic rats

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Abstract

Objectives: The purpose of the present study was to examine the effect of four-week resistance training on liver glycogen and lipid profiles of male rats. Wistar male rats obtained from the Pasteur's Institute were used in this research.

Materials and methods: Thirty two Wistar rats were randomly assigned into 4 equal groups (n=8) of non-diabetic-control, non-diabetic-training, diabetic-control and diabetic training groups. The resistance training included ascending a one-meter ladder with a weight hung from rats' tails. The ladder consisted of 26 steps in an 80° position. In order to assign proper weight, the rats were weighed every 4 days. 2 times ascending and descending the ladder without any weight following and preceding each training session were considered for warming up and cooling down. The resistance training was conducted three times per week.

Results: the result of analysis showed that there was significant difference between the liver cholesterol levels of non-diabetic control and non-diabetic trained rats. In addition, there was significant difference between the non-diabetic trained and diabetic controlled rats. Furthermore analysis indicated that there were significant differences between the liver glycogen of diabetic trained and diabetic controlled rats ($p<0.05$).

Discussion and Conclusion: It was concluded that four weeks of resistance training had no significant effect on the liver lipid profile of diabetic Wistar rats whereas resistance training program had a significant effect on the their liver glycogen.

Key words: resistance training, diabetes, liver lipid profile, liver

Introduction

Diabetes mellitus has long become a worldwide health concern predisposing to ever-increasing cardiovascular mortality and morbidity (1). Lipid abnormalities significantly resulted in the reduced risk of cardiovascular disease and other morbidity in diabetics (2).

Glycogen synthesis in liver and skeletal muscles are interrupted during the diabetes (3). Therefore, muscle and liver glycogen content is decreased during this condition (4). The disturbances in lipid profile is one of the common problems associated with diabetes. Approximately 40 percent of diabetic patients face this problem (5). Diabete results in increase of cholesterol, triglyceride and LDL level of serum (6). High level of cholesterol in blood is a major risk factor for coronary heart diseases. Acute deficiency of insulin results in release of fatty acid from the fat tissue that increases the LDL particles full rich in cholesterol (7,8).

In one study including 350 male and female subjects with and/or without fatty liver, it was found that the subjects who performed resistance training experienced lower outbreak of fatty liver (9). This is consistent with the results obtained from in vivo studies showing resistance training to reduce fatty liver (11), diet-induced reduction in fatty liver (10), and liver lipid content following weight loss period (12,13). In addition, resistance training reflects variations in vascular adipose tissue (14,15). The findings imply that resistance training may be considered a useful approach in order to prevent or decelerate liver lipid atrophy. Nevertheless, there was no report about effect of resistance training on liver glycogen and lipid profile concentration in diabetic subjects.

Considering the effect of resistance training on elevation of muscular mass and strength as the biggest blood glucose-consumer, the present study aimed at evaluation of effect of a period of resistance training on liver glycogen and lipid profile concentration in diabetic rats and find an answer to the question whether four weeks resistance training affect liver glycogen and lipid profile of diabetic rats.

Materials and Methods

Rats maintenance and feeding

Thirty six male rats from the strain Wistar (12 week-old) with average weight of 288±22 gr were adopted. The animals were bought from Pasteur's Institute(Tehran, Iran) and were kept in special flexi-glass (43×27×25 cm) with net doors and in controlled ambient status (temperature: 22±2°C; light/dark period: 12:12 hr). Animals were fed a pellet rodent diet ad libitum in domesticated animals' foods production units and had free access to water. The food used in the present study was produced in Khorak-Dam-Pars Co. Also, water was given in special 500ml bottles. After adaptation to the lab environment, the rats were randomly divided into 4 groups: [1] non-diabetic control; [2] non-diabetic training; [3] diabetic control; and [4] diabetic training.

Study design

The resistance training included ascending a 1m ladder with weights hung from animals' tails. The ladder had 26 steps in 80° position. The training program began 7 days following the injection. The rats were weighed every 4 days in order to assign accurate weight. It should be noted that this was the first model of using resistance training for diabetic rats. The training intensity was chosen to exert the minimum damage to muscular fibers. Furthermore, resistance training along with minimizing the eccentric contractions helps reducing the damage. In the present study, training program consisted of primary (load increase) and main (fixed load) stages each lasting 2 weeks. Before training onset, the animals were taught to ascend the ladder. In order to reduce the stress level, electrical shock, air pressure, cold water, and food reward or limitation were not adopted. The animals' tails were only touched and rubbed to stimulate training. In primary stage, training intensity (weights and number of iterations) gradually increased, i.e. the first time consisted of 2 parts each with 6 iterations with a weight around 30% of animal's weight. The animals were allowed to rest for 1 min between the iterations and 3 min between the times. In the beginning of main stage (third week), the animals were able to perform training in 3 sessions with weights weighing 100% of their body weight. The intensity was kept unchanged until the end of training program. Training was performed three times a week in alternate days. 2 times ascending and descending the ladder without any weight following and preceding each training session were considered for warming up and cooling down.

Diabetizing method

The diabetic condition was induced by one-time inter peritoneum injection of soluble *streptozotocin* dissolved in citrate 0.1 percent molar at the rate of 55mml/kg of body weight. For the non-diabetic animals, equal volume of buffer citrate was injected. Five days following the injection, blood samples was drawn from the tail of animals and through the enzymatic method, glucose oxidase was assessed. The concentration of 250 mm/dl glucose or higher amount in blood was defined as being diabetic.

Tissue biopsies

Glucose concentration was measured through enzymatic-chromatographic method with glucose oxidase technology and by the use of glucose kit (Pars Azmoon Co., Iran). Coefficient of variation and sensitivity of the measurement method were 1.8% and 5 mg/dl, respectively. In order to measure the concentrations of HDL-C and Cholesterol as well as the concentration of triglyceride, enzymatic-photometric (Pars Azmoon Co, Iran) and enzymatic-chromatographic (Pars Azmoon Co, Iran) methods were employed. Friedvald et al. method was used to measure the concentration of LDL-C. Inter-assay coefficient of variation and sensitivity of the method for HDL-C, cholesterol, triglyceride, and NEFA were 2.2% and 1 mg/dl, 1.2% and 3 mg/dl, 2.4% and 1 mg/dl,, and 3.1% and 0.1 mg/dl. Glucose was measured via enzymatic-chromatographic methods through

glucose oxidase technology by use of glucose kit (Pars Azmoon Co, Iran). Intra-assay coefficient of variation and sensitivity of the method were measured to be 2.3% and 5 mg/dl.

Statistical analysis

Statistical analysis was performed by using analysis of one-way variance (One-way ANOVA) and LSD post hoc test was employed to locate the differences. Whenever the normality assumption was violated, Kruskal–*Wallis* test was used. All the analysis was performed by SPSS:PC 16.0. The level of significance was set to 0.05.

Results

The result of analysis showed that the there was a significant difference between the liver cholesterol of the animals in the experimental groups after the competition of the training protocol ($p<0.05$). LSD post hoc test indicated that the differences were between the liver cholesterol of non-diabetic control vs non-diabetic trained rats and between the non-diabetic trained and diabetic controlled rats ($p<0.05$) (Figure 1).

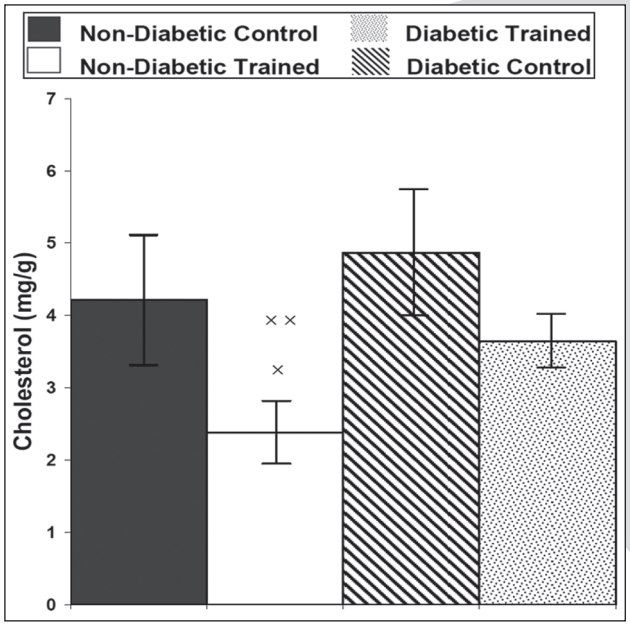

Figure 1. Comparing the liver cholesterol of the experimental groups

Abbreviation: NDC= non-Diabetic Control, NDT= non-Diabetic Trained, DC= Diabetic Control, DT= Diabetic Trained

** Significant difference between NDT and DC groups.*

*** Significant difference between NDT and NDC groups.*

The result of analysis indicated that there was no significant difference between the livers triglyceride of the experimental groups following the participation in the training protocol ($p>0.05$) these results are presented in Figure 2.

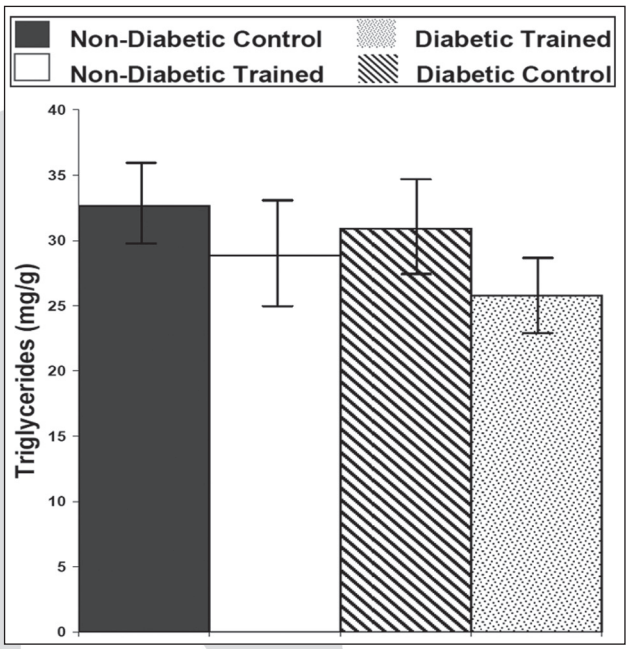

Figure 2. Comparing the liver triglyceride of the experimental groups

Abbreviation: NDC= non-Diabetic Control, NDT= non-Diabetic Trained, DC= Diabetic Control, DT= Diabetic Trained

The result of Kolmogorov-Smirnov test showed that the distribution of liver HDL-C was not normal; therefore, Kruskal-Wallis test was used to test the hypothesis. Further analysis indicated that there was no significant differences in liver HDL-C of the subjects in all the experimental groups ($p>0.05$). These results are presented in Figure 3. In addition, the result of Kruskal–Wallis test indicated that there was no significant difference between the mean values of blood glucose in trained diabetic rats versus the control diabetic rats ($p>0.05$) (Figure 4). There was a significant difference between the mean values of liver glycogen of diabetic Winstar rats versus the controlled diabetic ones ($p<0.05$) (figure 5).

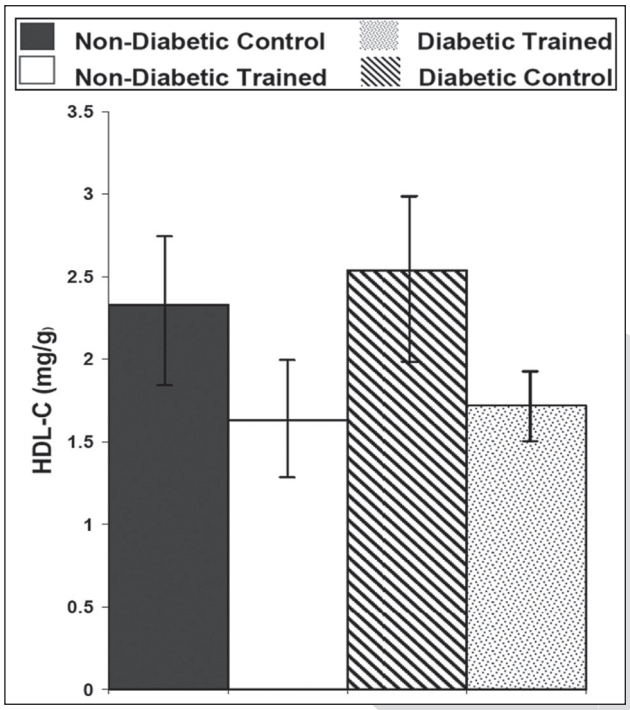

Figure 3. Comparing the liver HDL-C of the experimental groups
Abbreviation: NDC= non-Diabetic Control, NDT= non-Diabetic Trained, DC= Diabetic Control, DT= Diabetic Trained.

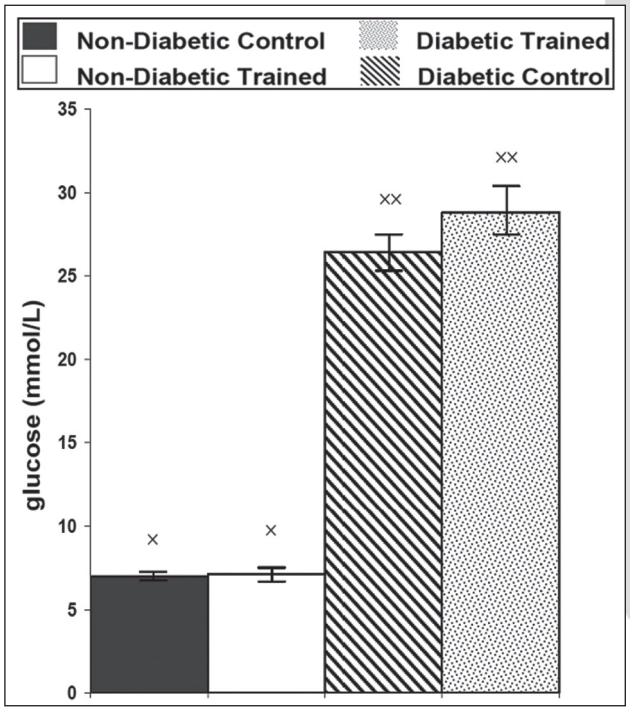

Figure 4. Comparing the liver Glucose of the experimental groups.
Abbreviation: NDC= non-Diabetic Control, NDT= non-Diabetic Trained, DC= Diabetic Control, DT= Diabetic Trained.
**Significant difference between NDT and DC Groups.*
***Significant difference between NDC and DT Groups.*

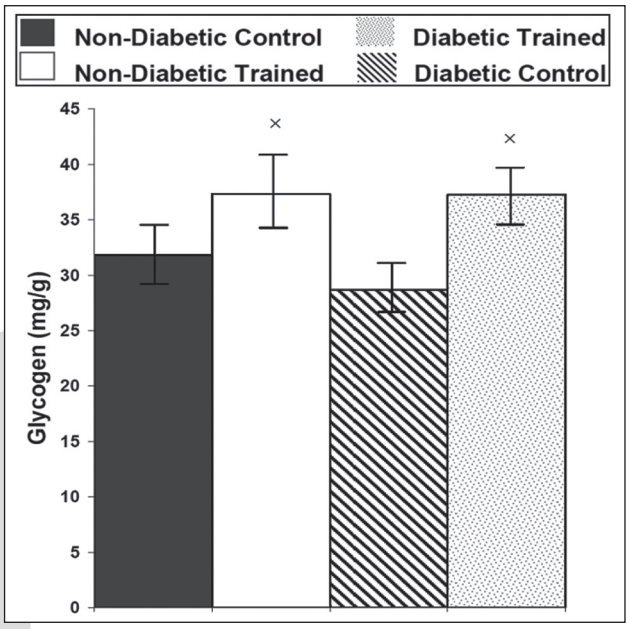

Figure 5. Comparing the liver glycogen of the experimental groups
Abbreviation: NDC= non-Diabetic Control, NDT= non-Diabetic Trained, DC= Diabetic Control, DT= Diabetic Trained
**Significant different between NDT and DC groups.*
***Significant different between DT and NDC groups.*

Discussion

The purpose of the present study was to examine the effects of the resistance training on liver glycogen and lipid profiles in Wistar rats. The initial hypothesis was partially confirmed. While four weeks of resistance training resulted in improvement of liver lipid profile, however, these changes were not significant in diabetic training group. Nevertheless, significant differences were detected between non-diabetic trained group and diabetic and/or non-diabetic control group. In this regard, research examining the effects of resistance training is limited since most of the researches employ endurance exercises. Considering the pathogenesis and pathophysiology of type 2 diabetes, the role of resistance training in the treatment regimen needs to be determined (15,16). Type-2 diabetes is mainly a disorder of aging and the decline in muscle mass with aging is associated with a decline in metabolic function, thus, supporting the usefulness of resistance training in the diabetes treatment regimen.

There are reports claiming that resistance exercise (mainly acute) affect lipid metabolism while (17). showed that moderate acute resistance exer-

cise (73% of 1 RM) caused favorable change in lipid profile, increasing HDL-c and its sub fractions (HDL2 and HDL3) compared with high intensity resistance exercise (92% 1 RM). Such result may be attributed to the differences between the loading volume. Thus, total energy expenditure may, at least partly, determine lipid metabolism change following physical activity.

David et al conducted a research in which diabetic overweight and fat men and women participated in progressively increasing resistant training with and without mean weight reduction to examine the changes of serum insulin, glucose and lipids. These authors found that there was no significant change in these serum components. The result of the present research (HDL, TC and TG) in not in agreement with findings of that research in the diabetic control and training groups (17).

Sijie and Li also studied the profiles of lipids in chronically type-2 diabetic patients and showed improvement in these profiles (18). These results are also in contradiction with the result of the present study probably due to the different types of training program and the duration of the protocol.

Dyslipidemia has resulted in mortality of the type-2 diabetic patients and this is the general pattern in type-2 diabetic patients with high triglyceride, LDL, and low HDL (19).

Agraval et al reported that a significant improvement in glycemic condition and lipid profiles occurred following the participation of the patients in Yoga exercise. They reported that a significant decrease in total glyceride, triglyceride and lipoprotein with low density and increase in high density lipoprotein was the result of three month of participation in the exercise program (20).

David et al demonstrated that high volume resistance training with 8 to 12 maximum repletion resulted in desirable improvement in blood lipid profiles as well as increase in local HDL-C, but no significant changes occurred in total cholesterol (17). Welihinda et al showed that intense resistance training has no significant effect on the secretion of LVDL-C and TG. These results support the finding of the present study that recorded a 26 percent of decrease in VLDL-C and TG (4).

George and associates conducted a research to examine the effect of progressively increasing resistance training on lipid and lipoprotein profiles in adult subjects. The result of their study showed that HDL-C and TG of serum decreased following the completion of the exercise program (17).

Niel and Will noticed that all of these training, particularly the combination type (strength and endurance), produce beneficiary effect on decrease of glucose level and the range of these effects ranges from low to moderate (21). However, in the present research, the resistance training produced no significant change on glucose level of diabetic rats probably due to the short course of training, its intensity or the use of steritozeocin to induce diabetes high enough in the diabetic control group masking the difference compared to the healthy control group.

David and Goodpastar examined the effect of exercise on glucose homeostasis of type-2 diabetes and concluded that it is not possible to determine whether there is an association between the volume or intensity of exercise and improvement or control of glucose in type-2 diabetes, it is conceivable to observe no significant change in glucose level following the participation in an exercise protocol like the one employed in this research (22).

Carman et al examined the effect of resistance training on improving glycemic control in elderly adults afflicted to type-2 diabetes and observed that progressively increasing resistance training resulted in improvement of muscle glycogen store (23).

Jane et al examined the acute effect of aerobic exercise versus resistance training (3 sets of 7 movement with 8 repetitions for 45 minutes) in type-1 diabetic patients and concluded that plasma glucose decreased in both aerobic and resistance training (24). This finding is not in agreement with the finding of the present research in regard to the decrease in plasma glucose probably due to the acuteness of their training session.

Crespilho et al studied the effect of exercise programs on immune system of diabetic rats and reported that aerobic sport activities result in increase of glycogen level of liver and decrease of cholesterol and triglyceride of liver of artificially induced diabetes by sterotozin in rats (25).

In conclusion, the results of the present research showed that resistance training program may improve liver lipid and glycogen status in diabetic patients and duration and intensity of the training play a prominent role. However, further resear-

ches are required to obtain more precise results about diabetic subjects. In addition, the resistance training can be a useful program and have positive effect on liver glycogen and lipid especially in diabetic population.

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Barrier of Distance and Transportation to Access Antenatal Care in Muzaffarabad Azad State of Jammu &Kashmir

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Abstract

Objective: To find out dependence of antenatal care utilization with transportation barriers in muzaffarabad.

Methods: Descriptive cross sectional survey was conducted in Muzaffarabad Azad State of Jammu & Kashmir during the period of June to December 2013. study was conducted over 500 reproductive age (15-49) women who had history of at least one child birth. Sample was withdrawn from population through systematic random sampling technique. Univariate (frequencies) and multivariable logistic regression model was used to find out dependence. data was analysed in SPSS version 20.

Results: 53.2% women have not received antenatal throughout their pregnancy. 51.2% women confronted problem of unavailability of transport at night similarly 50.4% women belonged to study area were of view that above 40 minutes are required time to reach health facility. Type of road is associated with uptake of antenatal care use (p=0.00). Odds of distance from home to road above 5Km are 0.51 lower with respect to antenatal care utilization than of reference category. Similarly availability of motor vehicle to reach to road is 0.15 times lower in utilization of antenatal care.

Conclusion: non availability of transport, irregular pattern of public transport and long travel time are significantly associated barriers in utilization of antenatal care. Access barrier can lead to differences in health utilization pattern of women. batter access to health facility is significantly associated with antenatal care utilization.

Key words: Antenatal Care; Odd Ratios; Confidence Interval; Muzaffarabad

Introduction

Transportation is well recognized obstacle in health care use by expectant women but this is understudied in terms of particulars required towards addressing direct health and transportation strategy interventions. This research explains the issues related with transportation faced by women in pursuing health care during pregnancy. Due to geographic characteristics of Kashmir majority of women are disadvantaged in getting adequate care during pregnancy. Health barriers are defined as the many states, circumstances, or measures that make it hard or restrict a woman from effectively obtaining maternal health care. Prevention in order to be truly preventive must be antenatal care (Ballantyne, 1902).Antenatal is perfect example of preventive medicine (Great Britain; social services committee, 1980). Antenatal care is additionally expected to be functional if women start reception of care in the first trimester of conception and persist to obtain care all through pregnancy, according to accepted principles (US department of health and Human Services, 2010). Antenatal care (ANC) is a continually used health inspection that has the likelihood of lessening the incidence of perinatal morbidity and death by treating medical situation, identifying and reducing apparent risks, and helping women to deal with behavioural factors that add to poor outcomes. (US department of health and Human Services, 2010) whereas The perinatal phase starts at 22 completed weeks and ends seven finished days after birth. Maternal and perinatal health is directly related. Perinatal death refers to the number of stillbirths and deaths in the first week of life that is early neonatal mortality (1).

Antenatal care services assist women by recognizing medical problems related with conception or morbidity that has strength to harmfully affect

the pregnancy. During antenatal visits, women get advantage from a variety of interventions, including the provision of folic acid/iron supplements, counselling about healthy lifestyles and TT immunization reports to defend newborns against neonatal mortality(2). Azad state of Jammu & Kashmir is patriarchal society where women face discrimination in all walks of life .women are not self sufficient in taking decision, plus decision to utilize maternal health services. Transportation barriers and difficulty in getting vehicles raised seeing that the key problem among mothers who did not accomplish the least needs of four antenatal care services during their conception period. while health care services are directed at the state level, much also depends on state administration and organization skills

Distance to a health facility plays a essential role in health service accessibility. facility delivery for a birth within 1 kilometre of a comprehensive health facility are over 10 times higher for a birth whose closest facility is 20 kilometre away or above(3).

Every year there are more than 500 000 maternal deaths worldwide while an estimated 4 million newborns die, and another 3 million babies are stillborn (4). Once the barriers to care are identified, it becomes possible to take the next steps to increase access to maternal health care. Creating solutions and strategies to improving access is a necessary component of combating this problem. Pakistan has gained improvement in these health indicators but still it has high fertility, inadequate institutional deliveries, under utilization of antenatal care and family planning methods. Problems of conception serve as most important reason of casualty as well as disability in favour of childbearing mothers in much of the developing world. Of the estimated 529,000 maternal deaths each year, 99% take place in the developing world (5). This condition is even worst in Muzaffarabad Azad Kashmir due to geographic location as this is hilly mountainous area which makes it difficult to pursue medical advice due to transportation problem and conservative socio cultural fabric of society where women is under collective decision of husband and other family members in pursuing medical advice during and after pregnancy. Women constitute half proportion of population in muzaffarabad. Their proportion itself highlights significance of issue to be studied to bring about development in all walks of life. external barriers faced by women in utilizing health care during pregnancy is non availability of transportation(6). common barriers faced by women are transportation shortage and long distance from home to health facility (7). Mothers who access antenatal care by walking are to be expected to avail below four antenatal care appointments compare to mothers using transportation(8).travel time to facility is major hindrance in receiving antenatal care. For rural women transportation is main barrier restricting them from hospital care during pregnancy and child birth(9). There are three main reasons of non utilization of maternal health services that are economic, awareness regarding women as well as child wellbeing & access to health care (10).

Methodology

Research Design: descriptive cross sectional research was conducted in muzaffarabad. Muzaffarabad was selected to study defined topic because of vulnerability of mountainous area. Another reason to select area is that apparently there is no study on transportation barrier in utilization of antenatal care in muzaffarabad.

Respondents: women of reproductive age group (15-49) who had history of at least one child birth is included in study to better explore their experience of transportation barrier. Representative sample of systematic sampling technique was used to draw sample from population.

Instrument /Tool: A close ended questionnaire was developed including all the variables like transportation availability, road type, distance to health facility and utilization of antenatal care. The questions were developed based on the existing literature relevant to barriers to accessing maternal health care. Data was collected during the period of June to December 2013.research ethics were followed by ensuring privacy & anonymity of information provided by respondents

Measures /Variables: present study used one dependent variable that is utilization of antenatal care or not and independent variables were availability of transportation at night, distance from road to health facility, road type, distance from home to road. Some independent variables were recoded in

to binary categories because responses in previous category was negligible. Recoded variables are distance to health facility (< 40KM & > 40 KM), distance from home to road (< 5KM & > 5KM) ,way to reach road (walk & motor vehicle).

Data Analysis: Data were entered & analysed using SPSS version 20. univariate (frequencies) and multivariable logistic regression model was used to find out dependence of transportation factors in utilization of antenatal care in muzaffarabad.

Results

Table 1 indicates that 67.4% of women were above 30 years similarly 95.4% husbands were above 30 years. 53.2% have not received ante-

Table 1. Distribution of Respondents according to Maternal Health Care Utilization (N=500)

Characteristics	Variable	Frequency	Percentage
Age of women(Years)	Less than 30	163	32.6
	Above 30	337	67.4
Age of Husband(Years)	Less than 30	23	4.6
	Above 30	477	95.4
Received ANC	Yes	234	46.8
	No	266	53.2
Current Maternal Phase	Non Pregnant	335	67.0
	Pregnant	96	19.2
	Post natal phase	69	13.8
Parity	1	37	7.4
	2	145	29.0
	3	53	10.6
	4 & above	265	53.0
Timing of first ANC check up	Not received	266	53.2
	1st trimester	137	27.4
	2nd trimester	38	7.6
	3rd trimester	59	11.8
Number of ANC visits	No visits	228	45.6
	1-2	65	13.0
	3-4	75	15.0
	5-6	84	16.8
	7 & above	48	9.6
Complication during pregnancy	No complication	286	57.2
	Haemorrhage	125	25.0
	Increased BP	82	16.4
	Diabetes	7	1.4
ANC Attendant	Doctor	237	47.4
	Nurse/Midwife	35	7.0
	TBA	214	42.8
	No one	14	2.8
Place of ANC	Home	90	18.0
	Private hospital	260	52.0
	Public hospital	150	30.0
Reason of first ANC check up	Had problem	275	55.0
	Just for check up	225	45.0
In complication women seek care from	Private hospital	268	53.6
	Public hospital	56	11.2
	TBA	176	35.2

natal care at all during whole conception period from expert medical professional whereas 27.4% received care in first trimester of pregnancy. as far as number of ANC visits are concerned 45.6% had not received ANC as compared to 16.8% attended 5-6 ANC visits. 57.2% women had no complication during pregnancy. 47.4% respondents received ANC care from doctor where as 42.8% received care from traditional birth attendant. 52% women belonged to study area received care during pregnancy from private hospital. 55% of women received care during pregnancy because that had medical problems. 53.6% seek medical advice in case of emergency from private hospital.

Table 2 indicates that 46.4% women belonged to area from where distance to main hospital (Combined Military Hospital & Abbas Institute of Medical Sciences) were less than 40 kilometre. huge majority of respondents (76.2%) were having good road type (pakka) as compare to rocky road type (23.8%).bit higher than half of respondents were residing 6-94km far from main road. similarly due to greater distance from main road majority of women (38.2%) get to road by walk. almost all sampled population (97.4%) utilize motorized transport as compare to negligible proportion (2.6%) who did not use motorized transport. huge majority of women (91.6%) had not used non motorized transport. bit higher than half (51.2%) confronted problem of unavailability of transport at night similarly slight higher than half (50.4%) women belonged to study area were of view that above 40 minutes are required time to reach health facility.

Data presented in table 3 indicates that odds of above 40 kilometre distance to district head quarter hospital is 0.02 times lower in utilization of antenatal care and significantly associated with ANC utilization. Type of road is associated with uptake of antenatal care use (p-0.00). Odds of distance from home to road above 5Km are 0.51 lower with respect to antenatal care utilization than of reference category. Similarly availability of motor vehicle to reach to road is 0.15 times lower in utilization of antenatal care than of those who go by walk to road. Motorized transport is significantly associated with antenatal care utilization. Odds of transport availability at night are 0.03 times lower and are strongly associated with uptake of antenatal care that area where transport is not available at night utilization of antenatal care is 0.03 times

Table 2. Frequency Distribution of respondents according to transportation barriers (N=500)

Characteristics	Variable	Frequency	Percentage
Distance to DHQ hospital	Less than 40 km	232	46.4
	41-94km	128	25.6
	95 km & above	140	28.0
Road type	Mainly kutcha	119	23.8
	Mainly pukka	381	76.2
Distance from home to road	Less than 5 KM	199	39.8
	6-94km	251	50.2
	95 KM & above	50	10.0
Way to reach road	Walk	191	38.2
	Rickshaw	126	25.2
	Motorbike	103	20.6
	Private vehicle	80	16.0
Motorizes Transport	Yes	487	97.4
	No	13	2.6
Non motorized transport	Yes	42	8.4
	No	458	91.6
Transport availability at night	Yes	244	48.8
	No	256	51.2
Required time to reach health facility	30-40 Minutes	207	41.4
	Above 40 minutes	252	50.4
	Don't know	41	8.2

Table 3. Transportation Barriers Against Antenatal Care Utilization

Characteristics	Variable	OR*	95% C-I**		P Value***
			Lower	Upper	
Distance to DHQ hospital	Less than 40 km	1.00			0.000
	Above 40 KM	0.02	0.01	0.037	
Road type	Mainly pukka	1.00			0.000
	Mainly kutcha	0.00	0.00		
Distance from home to road	Less than 5 KM	1.00			0.000
	Above 5 KM	0.51	0.35	0.73	
Way to reach road	Walk	1.00			0.002
	Motor Vehicle	1.75	1.21	2.53	
Motorized Transport	Yes	1.00			0.000
	No	0.00	0.00		
Non motorized transport	Yes	1.00			0.407
	No	0.63	0.29	1.36	
Transport availability at night	Yes	1.00			0.000
	No	0.03	0.02	0.05	
Required time to reach health facility	30-40 Minutes	1.00			0.000
	Above 40 minutes	0.01	0.00	0.02	

*1.00=reference category, *Odd Ratio, **Confidence interval, ***p value significantly associated*

lower. Similarly odds of required more time to reach to health facility is 0.01 times lower that is more distance to reach to health facility has negative relationship with utilization of antenatal care

Discussion

Non availability of transport is dominant barrier in utilizing antenatal care. Along with many other socioeconomic barriers lack of access to transportation and cost is factor restricting women in pursuing health care during pregnancy. Distance to health facility determines attitude of women toward health seeking behaviour as health seeking behaviour of women during pregnancy are negatively related that is with increase in distance to health facility likelihood of receiving antenatal care get decrease (11). Further more uneven pattern of public transport is a different hurdle in utilization of health care as women has to perform domestic job so it becomes difficult for them to wait for public transport for indefinite time.

Long travel and deficient resources to travel are main factors associated with health care utilization during pregnancy along with many other social barriers (12).

Due to easy availability of traditional birth attendant and non availability of transport women are forced to seek treatment from traditional healers even though they have awareness about utilizing expert medical advice .infrastructural resource is important in developing mind set of people regarding care utilization. Additionally most of main roads are under construction that's why women need to wait for long hours to reach to health facility due to this delay they prefer to seek care from traditional healer. motor vehicle facility to health facility are not consistent ,bus stops are in good conditions. Furthermore strikes of drivers further delays appointments during strike days(13).

People who live in rural areas or in hilly areas face transportation as major hurdle in seeking medical advice additionally poor quality of roads further restrict them from seeking health care. Affordability is also preventing them as people cannot afford private transport.

Conclusion

Along with many socio economic and cultural barriers transportation is dominant barrier in muzaffarabad. Due to geographic location of azad Kashmir women cannot receive antenatal during pregnancy due to non availability of roads & transport. There is need to appoint trained medical professionals(doctors and nurses) in remote areas

of muzaffarabad as 80% population is living in rural areas from those areas access to health facility is not possible for every women during pregnancy so trained health workers doctors & nurses can provide them medical care.

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Is social capital as a predictor of quality of life in multiple sclerosis patients?

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Abstract

Background: The bulk of existing research on patients with multiple sclerosis (MS) focuses on prediction based on medical (e.g., diagnosis, treatment) and personal (e.g., age, comorbidities, health behaviors) variables. But it seems that, so far, social-contextual factors such as the level of social capital and their relationship with quality of life in MS are not investigated. The aim of this study was to determine the relationship between social capital and quality of life (QOL) in MS patients registered with the MS Society of Iran in 2012.

Methods: This cross-sectional study was performed on 172 patients of the MS Society of Iran (resident in Tehran) for 10 months. The convenience sampling method was adopted and data collection tools were quality of life (MSQOL-54) instrument and World Bank Social Capital (SC-IQ) questionnaires. Data were analyzed by Pearson's test of correlation, one-way ANOVA, two-sample t-test, and multiple regression.

Results: The mean age was 34/8±9/6 years. The analysis of the 6 dimensions of social capital questionnaire showed that the highest mean score was for the membership of the groups and associations (63/3±15/3) and the lowest score was in social trust dimension (44/3 ± 13/7). Multiple regression results showed a positive significant relationship between quality of life and social capital (P<0.001).

Conclusions: The findings suggest that increasing social capital can promote QOL of MS patients, and vice versa. Therefore, increasing the individual components of social capital not only can lead to the society's benefit from their capabilities but it also has an important role in physical and mental health which in turn improves patients' QOL.

Key words: Social Capital, Quality of life, Multiple Sclerosis, MSQOL-54

Introduction

Multiple sclerosis (MS) is a chronic, progressive, and very common disease which is caused by demyelination of the central nervous system and results in a decreased personal and social performance (1).

Currently, more than 1.3 million people worldwide are infected with the disease (2). Recent studies in the Middle East and Iran suggest a high and increasing prevalence of MS in this area; the incidence has increased significantly from 0.68 in 1989, to 2.93 in 100 thousand in 2008 (3).

The complications of the disease limit the patient's health-related activities which increases secondary complications that restrains their independent life and adversely affects their QOL (4). Clearly, one way to enhance QOL is to identify factors affecting it.

So far, the majority of medical studies on MS patients' QOL have focused on predicting medical factors (eg. diagnosis, disease progression and treatment) (5-8) and personal factors (eg. age of onset, gender, family history, social and personal support, socio-demographic characteristics, comorbidities, health behaviors, and other confounding factors) (9,12-15) rather than broader social-contextual factors.

It seems that there are no studies investigating the correlation between social-contextual factors and the patients' QOL. Needless to say, like any other chronic illnesses, their ability to live with a high QOL, depends on their social environment (16). Characteristics of the patients' social envi-

ronment and their social interaction level; like understanding the level of social cohesion and trust in their neighborhood or the availability of local-social organizations and their social relations can affect the patients' QOL (17). In other words, quantity and quality of social relations and interactions can effectively change QOL (both the objective and subjective indicators).

Investigating social relations by means of social capital has added a new insight to this matter (18). Despite diverse definitions, social capital is generally defined as the level of social cohesion, cooperation, trust, interactions, mutual understanding and commitment among individuals in a social group (19-20).

Social capital has two main dimensions: cognitive and structural. Cognitive dimension involves: norms, values, attitudes, beliefs, trustworthiness, reciprocity, social cohesion, and social commitment among social groups (19-20).

Structural dimensions involves social support which accelerate human interactions and often refer to the density and strength of socialization ties, social involvement, organizations, social institutions and patterns of civic reciprocity (21-23).

Another useful conceptual distinction made in the field of social capital is the 3 types of social capital by Woolcock, He distinguished between: 1- Bonding social capital: which denotes ties between people in similar situations, such as immediate family, close friends and neighbors. 2- Bridging social capital: which encompasses more distant ties of like persons, such as loose friendships and coworkers. 3- Linking social capital: which reaches out to unlike people in dissimilar situations, such as those who are entirely outside of the community, thus enabling members to leverage a far wider range of resources than are available in the community (24).

Social capital is a very significant factor in health studies which effects people's health both at individual and social levels (25). National and international studies have shown that social capital dimensions and especially social trust has a major effect on human health which is not justifiable only by economic outcomes (26). Although studies have shown a positive relationship between social capital (measured by various methods), and population health in a variety of fields, namely, all causes of mortality (27); tools for health; diseases such as coronary artery disease (28); sexually transmitted disease (29); health-related behaviors (including smoking (30); exercise habits; diet and obesity (31-33); birth weight (34) child development (35); and mental health (22), they have not completely investigated advantageous effects of social capital on QOL of populations or vulnerable subpopulations like MS patients.

Therefore, studying social capital (cognitive and structural dimensions) and its effect on the QOL of MS patients is beneficial in two ways. On one hand, as MS patients spend more time at home, they have a greater reliance on local, social, and structural resources, and the local environment effects them more than other people (17); on the other hand, lack of social capital components for MS patients as a consequence of problems and disabilities caused by the disease, may decrease their membership of groups and associations and social involvement.

So, cultivation of patient's social relations and interactions not only brings benefits for the society by their abilities but also improves their mental and physical health as well as their QOL.

Since social capital is a key to improve MS patients' social-local involvement, their social status and QOL, this study aimed at assessing the relationship between social capital and QOL of MS patients in order to put a step forward in planning appropriate interventions for enhancing social capital and consequently their QOL.

Methods

The study population

A cross-sectional study of 172 patients was carried out based on convenience sampling method. All the patients were registered with MS society of Iran in Tehran and had MS for at least 10 months.

Data Collection Tools

The study tools included:

1 - A demographic questionnaire including the following questions: age, gender, marital status (single, married, widowed, divorced), education (illiterate, primary school, high school, university), years of successful education, employment status (employed, unemployed), household and individual average income, home size (approximate index of socioeconomic status), number of family

members (including the participant), number of children, home ownership (Yes, No), number of rooms (an objective indicator of social welfare), duration of residency , ethnicity, health insurance status, disease duration, age of onset, age at which the first symptoms of the disease appeared, the first symptom (sensory, motor, visual), having a family history of MS, other medical problems (Yes, No), current use of medication (corticosteroids), rehabilitation treatments, complementary and alternative therapies (yoga, exercise, meditation, nutritional therapy and herbal medicine, energy therapy and relaxation, acupuncture and acupressure) and their effectiveness (totally ineffective, partially effective, highly effective), progression type (relapsing remitting, primary and secondary progressive).

2 - Multiple Sclerosis Quality Of life-54 (MSQOL-54) questionnaire based on SF-36 which was previously validated in Iran (36). This 54-item instrument generated 14 subscales special for MS patients and 36 items on general QOL. The subscales were: physical function, role limitations-physical, role limitations-emotional, social function, health distress, and sexual function, satisfaction with sexual function, pain, energy, health perceptions, overall quality of life, and change in health, cognitive function and emotional well-being. Responses were likert type scale of 2 to 7 points (37). The overall QOL score of the patient was assessed by two composite scores, physical health and mental health. All 14 sub-scales and composite scores ranged between 0-100, the higher the score, the better the patients' QOL (38).

3 - World Bank integrated questionnaire for the measurement of Social Capital Integrated Questionnaire (SC-IQ) which is focused on applications in developing countries. The tool aims to generate valuable quantitative data on social capital of household (39-40). Iranian version of this questionnaire, developed by Nedjat et al. in 2012, contains 39 items and 6 dimensions: 1- inclusion in groups and associations, 2- social trust, 3- collective action and cooperation, 4-information and communication, 5-social cohesion, and 6- empowerment and political action and dimensions each of them addresses 7, 7, 3, 2, 12, and 8 questions, respectively, which respondents answer with a "yes" or "no". The scores range from zero to 100 and higher scores indicate higher social capital (41).

Data Collection

The investigator went to the MS society's office every day between 8 a.m. and 4: 30 p.m. for 5 months. Using the time before and after classes and physiotherapy sessions, and also the breaks the questionnaires were filled out. The procedure took about 25-30 minutes for each patient and questioners were filled avoiding the interviewer bias.

Prior to the interview, all the participants consented (written and oral) to participate in a research project after being informed of the scientific and scholarly aspects of the research.

Data Analysis

All data were analyzed with SPSS software version 20.0. Descriptive statistics like mean and standard error were calculated for social capital and QOL dimensions. To investigate the correlation between independent variables and dimensions of QOL, Pearson correlation, one-way ANOVA, and two-sample t-test were implemented (variables with a p-value< 0.2 entered regression model).

Finally, back ward regression was used to verify the effects of independent variables on social capital and composite scores of physical health and mental health, adjusted for confounders' effect. It should be pointed out that variables of age, average household, individual income, number of rooms, and home size were considered continues whereas variables of education, employment status, health insurance status, other medical problems, home ownership, age at which the first symptoms of the disease appeared, and complementary and alternative therapies were considered nominal. An alpha level of less than 0.05 was considered significant.

Ethical Considerations

Ethics Committee of Tehran University of Medical Sciences approved the study procedure.

Results

Demographic- Socioeconomic and Clinical Factors of Participants

According to descriptive statistics, the participants mean age was 34.76±9.56 years; the first symptom mean age was 24.92±8.73 years and mean disease duration was 8.1±6.4 years. Most participants were female (71.5 %), single (46.5%), holding a university

degree (48.5%) and unemployed (71.4%). Average individual income was 559607 ±324522 IRR and the mean home size was 102.6 ±52.9 m2. Disease progression of the patients in the study was mostly relapsing remitting type (85.3%). (Table 1)

QOL Dimensions

Analysis of all 14 dimensions of QOL showed that the highest QOL score was in social function (71.7 ±21.02) and the lowest score was in role limitations-physical (44.2 ±39.42) dimensions.

As for composite scores of QOL, the mean score for mental health (59.84 ±21.66) was slightly higher than the mean score for physical health (58.21 ±17.49), and the two scores were highly correlated (P<0.001). (Table 1)

Social Capital Dimensions

The MS patients in our study had, respectively, the highest and lowest scores on inclusion in groups and associations (63.3±15.3) and social trust (44.3±13.7) dimensions of social capital. As shown in table 1, the patient's mean score of social capital on inclusion in groups and associations, social cohesion and collective action and cooperation exceeds 50, but on information and communication and empowerment and political actions dimensions the scores were below 50.

A total of 166 (99.4%) participants were a member of social groups when MS association was taken into account, exclusion of the factor resulted in a low rate of 9% (9%) for the membership of groups. When patients were asked: "how much

Table 1. Descriptive statistics on composite scores of QOL (mental and physical health), social capital and its dimensions, age, home size, duration of residency, gender, education level, marital status and employment status of participants

Variables		Mean	SD	Min	Max
QOL composite score	Physical health	58.21	17.49	15.51	92
	Mental health	59.84	21.66	9.72	100
Social capital	Inclusion in groups and associations	63.3	15.3	21.43	96.43
	Social trust	44.3	13.7	6.82	75.00
	Collective action and cooperation	53.2	20.0	0.00	100.00
	Information and communication	52.6	20.0	588	94.12
	Social cohesion	54.1	10.3	25.71	80.00
	Empowerment and political action	47.1	16.7	1.14	94.32
	Total social capital	52.5	7.8	27.399	70.87
Age		34.76	9.56	16	66
Home size		102.62	52.88	40	450
Duration of residency		9.9	9.79	1	60
		n	**%**		
Gender	Male	49	28.5		
	Female	122	71.5		
Education	Illiterate	1	.8		
	Primary school	4	2.3		
	Middel school	9	5.3		
	High school	77	45		
	University	80	48.5		
Marital status	Single	79	46.5		
	Married	72	42.4		
	Widowed	2	1.2		
	Divorced	17	10		
Employment status	Employed	48	28.6		
	Unemployed	120	71.4		
Home ownership	Yes	107	62.6		
	No	64	37.43		

can you trust people?" only 4 (2.4%) answered very much, 9 (5.5%) said a lot, 62 (37.8%) said somewhat, 53 (32.3%) said a little and 36 (22%) said very little. Answering the question "in the last five years, has your trust level improved, worsened, or stayed the same?" 133 (79.6%) answered worsened, only 2 (1.2%) answered improved, 8 (4.8) believed it stayed the same, and 24 (14.4) answered "I do not know".

The trust level to different groups of people was measured based on a 5-point Likert scale (from 1, the lowest to 5, the highest), by which maximum and minimum trust level was for family members and strangers with a mean of 4.3 (SD=1.1) and 1.4 (SD=1.1), respectively.

Assessing the collective action and cooperation dimension, 78(48.8%) patients answered yes to the question: "in the past year have you joined together with others to address a common issue (e.g. helping someone in need)?" Another question was: "Suppose someone in the neighborhood had something unfortunate happen to them, such as a father's sudden death. How much do you think it is likely that people would help in this situation?" 16 (10.5%) said very unlikely, 36 (23.5%) said unlikely, 50 (32.7%) said somewhat likely, 36 (23.5%) said likely, and 15 (9.8%) answered very likely.

On information and communication dimension, the 3 main sources of information were "TV", "satellite" and "family, neighbors, and friends"; and 43.4% of respondents followed daily news, whereas 18.1% did not watch daily news at all.

On social cohesion dimension, 18 (10.8%) patients claimed that, in the past year, their car or house had been rubbed and/or they have been attacked, and according to 34.12% (49) of the participants 2 main issues causing gap and distinction between people were social status and wealth.

On empowerment and political actions dimension, 90 (54.5%) had voted in the latest election, and the mean score for the question: "how much can you change influential matters of your life?" was 3(SD=1.1), for which attainable answers were 1 (very little) to 5(very much).

QOL in Association with Demographic, Socioeconomic, Clinical and Social Capital Variables

To determine the factors affecting the 2 composite scores of physical health and mental health (adjusted for confounders), a multiple regression model was fitted with backward method.

Table 2 shows the list of variables that had a significant correlation with the outcome of interest and/or they couldn't be excluded from the model according to the maximum likelihood test. As it appears in the table, having other medical problems and use of corticosteroids (e.g. Betaferon, Rebif, and Avonex) is correlated with composite score of physical health; and inclusion in groups

Table 2. Assessing Effects of demographic, clinical variables and social capital dimensions on composite scores of physical and mental health using multivariate linear regression model with backward method

Dependent variable	Independent variables of final model	Standardized beta (β)	P-value	Coefficient of determination (R^2)
Composite score of physical health	Constant		0.08	0.13
	Education	0.24	0.06	
	Disease duration	-.25	0.05	
	Other medical problems	.32	**0.02***	
	Home ownership	-.27	0.06	
	Use of corticosteroids	.37	**0.01***	
	Social cohesion	.25	0.09	
	Empowerment and political acts	.23	0.09	
Composite score of mental health	Constant		0.82	0.49
	Inclusion in groups and associations	.38	**0.02***	
	Collective action and cooperation	-.53	**0.01***	
	Empowerment and political acts	.50	**0.01***	
	Other medical problems	.45	**0.006***	
	Complementary and alternative therapies	-.33	**0.03***	

Table 3. The effect of total social capital variable on quality of life in general

Variable	Regression coefficient	Test statistic (F)	P - value
Social capital (in general)	2.56	3.77	0.001>

and associations, collective action and cooperation, empowerment and political actions, having other medical problems and being under complementary and alternative therapies is correlated with mental health composite score.

Explained variance for regression model of outcome (QOL dimensions) by independent variables ranged from 0.13 for physical health to 0.49 for mental health composite scores.

Finally, Examining the relation between overall quality of life (dependent variable) and overall social capital (independent variable), we found a meaningful and positive relation (Table 3). In other words, any development and improvement in quality of life will lead to an increase in social capital.

Discussion

Studies have proved that improving social capital leads to a desirable level of QOL and well-fare. As Lin puts it, social capital is a beneficial (wealth, power, and fame) and significant outcome (physical and mental health, and life satisfaction) (42).

In this study, our goal was to determine MS patients QOL and its connections to social capital. Along with previous studies (17, 35, 43, and 44), our results showed that there was a positive correlation between social capital and QOL dimensions. In other words, increasing social capital can lead to a higher QOL for MS patients, and vice versa, hence the effect is reciprocal. According to the body of research in the field of social capital, it is possible to provide and promote social capital. Indeed, an understanding of concept and the importance of social capital. Then. obtain social skills which is necessary to create it in different environments. It can make a strong impact on quality of life in the general population, especially the vulnerable groups of society, such as may be seen in MS patients.

In accordance with findings of Ghaem et al. in Iran, analyzing composite dimensions of QOL showed that mental health score is only slightly higher than physical health score (45). This result points out the importance of mental components for MS patients' QOL. There is no evidence to show a relationship between mental disorders, like depression and stress, and physical component, such as severity of disability, in MS patients (46). As other studies have stated, in patients with less disability, physical component score of QOL was affected by mental components such as depression. This means that the mental dimension is the determinant factor of QOL even in MS patients with a very minor disability, and mental disorders like depression can cause challenges in coping with the disease (47).

In this study, inclusion in groups and social trust dimensions had the highest and lowest mean score, respectively; this result is in controversy with other studies on heart disease, diabetes, and obesity (28, 48). The maximum score of inclusion in groups and associations (structural dimension of social capital) appears to be due to factors that are similar between most study subjects; factors like gender (mostly female), age, marital status (being single), and education level (university degree).

"Gender" is one of the variable which influence the social capital and even on the type of membership in the community. So that most women participate as service members -in a charity, church, parent-teacher associations and cultural associations while men are interested in the activities of the political associations(20).

Age is another factor contributing in the development of social capital. Some studies have shown that there is a correlation between age and social involvements, such that between the ages of 18 to 29 memberships are singular, between 30 to 59 memberships are multiple, but declines after the age of 60 (20). Education also effects social capital. Membership in associations and social networks is higher among people with a college degree and generally educated people have more social relations, and there is even a direct relationship between years of formal education and social capital, which is partly due to the institutional environment of schools and universities encouraging participation in social networks (19). Therefore, the maximum score of inclusion in groups

and associations is justifiable with the fact that most patients in this study were women (73%), aged 30-59 (60%), single (60%), and holding a university degree.

In this study, age variable is not entered in none of the final models of the combination of physical and mental health domains. While age has a significant impact on quality of life in all domains in the general population of Tehran(49).

The reason may be the limited age range of the participants (16-66) and the fact that only 25 (5.5%) patients were over 50 years old.

Multiple regression analysis demonstrated a negative correlation between other medical problems and composite scores. Having other medical problems resulted in 0.32 and 0.45 Sβ (standardized regression coefficient[1]) decrease in the QOL score in physical and mental health, respectively. This is consistent with the work of Abdollahpour et al. (85) and is also an expected effect.

We observed a direct significant relation between the use of corticosteroids (e.g. Betaferon, Rebif, and Avonex) and physical health score. Using these medications yield to a 0.37-unit increase in physical health of the MS patients. This was expected because some studies have proved that corticosteroids are effective by decreasing the risk of relapse and Expanded Disability Status Scale (EDSS), which in turn promotes physical aspects of QOL (38).

In this study, complementary and alternative therapies affected mental health positively, so that mental health score increased by 0.33-units for MS patients receiving them. Our results confirm the results of other studies stating that complementary and alternative therapies improve QOL (56-61). Complementary and alternative therapies seem to affect QOL in MS patients by depletion of stress and depression, promotion of commonwealth (59) and mental health (60), regulation of adaptive immune and mental systems, and also modification of immune system (61).

The study also showed a significant correlation between inclusion in groups and composite score of metal health. One unit increase in the inclusion in groups' dimension increased the mental health score by 0.38-unit, and it was the same for empowerment and political act dimension with a higher coefficient than the inclusion in groups' dimension (Sβ = 0.50). These findings are not only consistent with other surveys, where the effect of social capital and social networks on mental health was studied, but also agree with the network theory. The network theory of social capital emphasizes on the social relation between people within groups and associations. Network theory fans believe that social networks are stress-buffering, by providing access to emotional support, friendship and opportunity to take part in significant social acts they improve one's self-esteem and capability to overcome problems which finally leads to mental health (68).

Collective action and cooperation was another variable that had impact on mental health, and the negative standardized coefficient suggested a negative and reciprocal relationship. This is in accordance with other researches reporting a poor, non-significant and even reciprocal relation between social capital and health (75-69).

Consistent with our results, Ziersch (74) and Ziersch & Baum (75) showed that social capital indicators have either a non-significant or reciprocal relation with physical health. It is worth mentioning that in health studies we often look for the positive aspects of social capital and the potential negative impact of social capital is overlooked. There are several theoretical arguments over the dark side of social capital in the literature (78-76). For example, Pearce et al. argued that intervention in the society in order to increase social capital may lead to inefficiency, resentment and excessive consumption of resources.

Based on such an approach, individuals may be criticized in the community and one's mental and physical health may be ignored at the macro level of social and economic policies (79). In this context, social capital in health studies is merely concentrated on micro level, holding the individuals responsible and entering them into social groups, many structural- and social factors are ignored that may accelerate the disease progression and have destructive effects on physical and mental health. Besides, the role of government and other private / public agencies is neglected (17).

The strength of this study was its contribution to social-epidemiology especially in the area of MS patients' QOL; as far as we know, it is the first

1 Standardized regression coefficient: enables comparison when more than one predictor variable is present.

study which evaluates the effect of social-contextual factors like social capital on MS patients' QOL. With over two decades of research, there is still no universal definition and/or measurement for social capital.

In addition to discussing the conceptual definitions, unlike other studies where only one or two items were considered for the measurement of cognitive- structural social capital, this study took advantage of a 6-item and 39-question tool to measure this multifaceted concept.

Finally, it is worth noting that MS society is not limited to a particular group of patients, and members are from different social- and economic classes, so there was no serious selection bias.

Study Limitations

Just like all cross-sectional studies, there was no evidence for causality. This study investigated the association between social capital and QOL, but still the exact nature of the biological mechanism or causal relationship is not clear. Although we have assumed that a higher social capital increases QOL, maybe a higher QOL results in more corporation and promotion of social capital in a society. Thus, longitudinal or in-depth qualitative studies may help to clarify the causal pathway.

Since this study was based on self-report measures, it is very prone to reporting bias; the reason is that measures were subjective and each interviewee had a different perception of the concept, particularly of the cognitive aspects (norms, values, attitudes).

One of the most important variables affecting QOL is social capital. Improved level of social capital, considering other effective variables fixed, can improve QOL.

The results of this study suggested that the mental health dimension of QOL and social capital of MS patients were positively correlated, so that an improved social capital resulted in QOL promotion, and vice versa.

Increasing the level of social capital components leads to community's benefit from their abilities in one hand, and promotion of their QOL in the other. For that reason, the authorities need to develop long-term plans according to population-based studies, and make fundamental changes in order to enhance QOL, and improve social capital and corporation of MS patients.

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Synchronic lesions - diagnostic difficulties: *Squamous* cell carcinoma, *Lentigo* maligna melanoma in patients with *Lepromatous Lepra* (A case report)

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Abstract

Leprosy is a chronic infectious disease involving the peripheral nerves, skin and other superficial tissues. In the presence of peripheral nerve involvement, trophic changes may occur in the extremities, predisposing to ulceration. The mechanism of malignant degeneration of chronic ulcers of leprosy is not well understood, but many factors have been reported, such as ulcers of long duration, chronic irritation, and trauma, and hygiene, environmental and genetic factors. Chronic ulcers and tumors are usually located in the foot, the most common site of neuropathic ulcers in patients with leprosy. Malignant neoplasm of the upper limbs is a lesion less common, but when it occurs, usually is located on the right hand. This matter aims to report an infrequent finding of squamous cell carcinoma (SCC) in a patient with lepromatous leprosy (LL). Data were collected from medical records and the skin was submitted to histopathological study with special stains. The findings were compared with the literature. Female, white, 51 years old, born in Pernambuco, northeastern Brazil, hypertensive and smoker, with no surgical history. She was in treatment for lepromatous leprosy six months ago. In the course of treatment, there was an improvement of the lesions, except a scaly plate located in the proximal portion of the dorsum of the right hand, which subsequently ulcerated. The histopathological evaluation was performed revealing squamous cell carcinoma, grade II, associated with lentigo maligna melanoma (LMM), overlapping the lepromatous leprosy. This report demonstrates the importance of early diagnosis of leprosy, in order to prevent the development of neuropathic ulcers that which can undergo malignant transformation. In addition, the SCC in chronic ulcers should not be considered very rare entity. No less important is the monitoring of minimal changes in chronic ulcers with serial biopsies when there is any change in the clinical course of the lesion.

Key words: Leprosy, Skin ulcer, Squamous cell carcinoma, Lentigo Maligna.

Introduction

Leprosy is a chronic, infectious and bipolar disease, determined by infection with ***Mycobacterium leprae and Mycobacterium leprosy***, characterized by the involvement of peripheral nerves, integument and other superficial tissues (Satish *et all.,* 2008; Han *et all*., 2008). It is primarily a granulomatous disease of the peripheral nerves and mucosa of the upper respiratory tract and the skin lesions are the primary external sign. Untreated leprosy tends to be progressive, causing permanent damage to the skin, nerves, limbs and eyes, often ulcerated. Contrary to the popular saying, leprosy does not cause loss of body parts, often. You can display decreased sensitivity, befallen secondary infections. These result from immunosuppression determined by the primary disease. Secondary infections, in turn, may result in tissue loss, culminating in ulcers, shortening and deformities, destruction of sequential cartilage (Jorge *et all.,* 2009). Has not been described in the literature the malignant transformation of lepromatous lesions. However, the synchrony with the skin neoplasm has been described in particular epithelial tumors (Ratoosh, Cohen, Trancoso, 1994; Sandhya *et all.,* 2010).

The mechanism of malignant degeneration of chronic ulcers in leprosy is not well understood, but many factors have been reported, such as ulcers of long duration, constant irritation, trauma,

hygiene, environmental and genetic factors (Zagne Bauk, 2006).

Chronic ulcers and tumors are usually located in the foot, which is also the most common site of neuropathic ulcers in patients with leprosy. Malignant neoplasm of the upper limbs is less frequent, but when it occurs, usually is located on the right hand (Barella, Blanco, Yamane, 2013; Gomes, Frade, Foss, 2007). No case of simultaneous lepromatous leprosy, squamous cell carcinoma and lentigo maligna has been described.

This paper aims to report a case of lepromatous leprosy observed simultaneously with squamous cell carcinoma and lentigo maligna.

Method

It is individual descriptive study, whose data were obtained from the medical records, from the outpatient Tropical Pathology, Faculty of Medicine, Federal University of Cariri, Barbalha, Northeastern Brazil. The flow of information was obtained through a project submitted to the Ethics Committee of the Faculty of Medicine, Federal University of Cariri. The authorization for the execution of the work was released by application to the trustee of the information and data, following all ethics recommendations of the research in humans. All data were tabulated in a text editor and spreadsheet of the Microsoft ®. Portal Capes collected updated bibliography in the database of Medline and PubMed; also piecemeal literature journals available on the web using as descriptors: Leprosy, Skin ulcer; Diagnosis; squamous cell carcinoma; Lentigo Maligna.

Case report

Admission - Female, white, 51 years old, born in Pernambuco, northeastern Brazil, hypertensive, smoker, no surgical history. He was in treatment for lepromatous leprosy six months ago.

History - Patient was referred to the outpatient clinic of tropical pathology with good general condition but was diagnosed with lepromatous erythematous s leprosy under treatment for six months. The various lesions in plaques erythematous on the trunk and limbs had improved, but an injury in high plaque, scaly on the dorsum of the right hand did not improve. It was a lesion in plate with 12 inches with central deep ulceration of 7 inches. There were no other complaints.

Evolution - Routine laboratory tests were normal. A biopsy with histopathology was requested. The incisional biopsy for histopathological evaluation revealed the macroscopic fusiform piece of clear skin, firm and elastic measuring 5.7 cm in its greatest diameter. Microscopy revealed a squamous cell carcinoma – SCC (positive for human pancytokeratine through the immunohistochemistry) grade II (Figure 2), associated with lentigo maligna melanoma - LMM (S-100 protein and HMB 45 positive), (Figure 4), both superimposed on lepromatous leprosy (Staining by WADE revealed many acid-fast bacilli), (Figure 3).

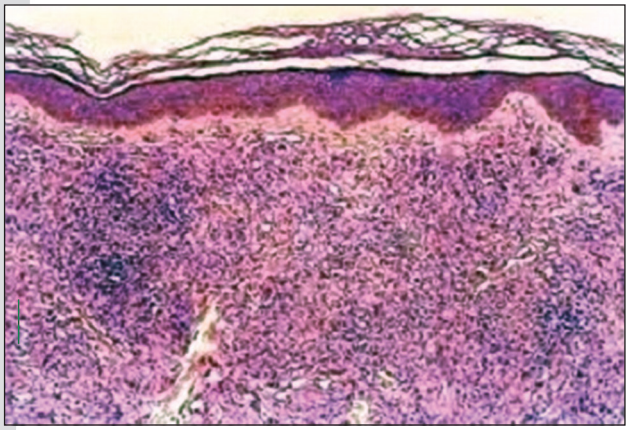

Figure 1. Overview

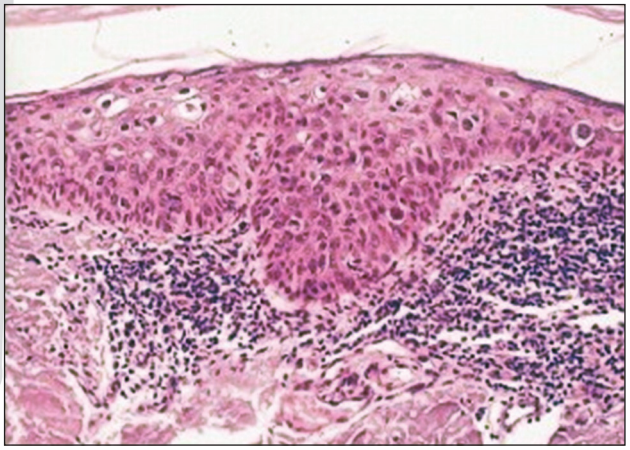

Figure 2. SCC

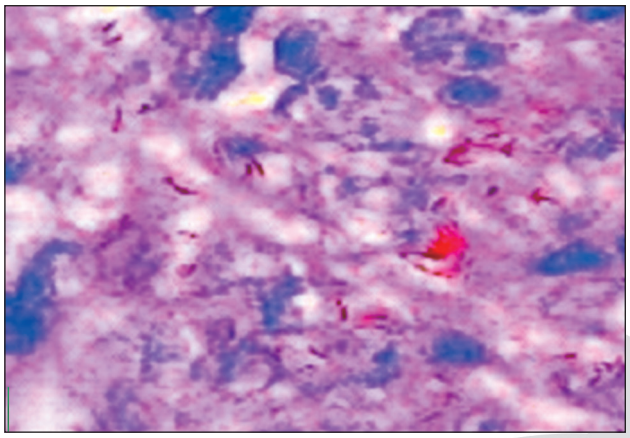

Figure 3. Acid-Fast Bacilli

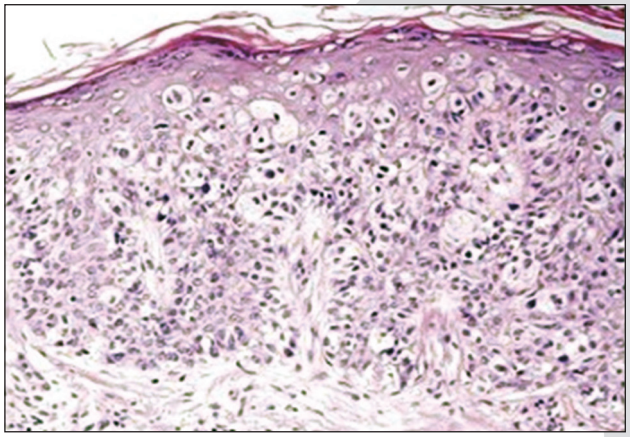

Figure 4. LMM

Result and discussion

Leprosy is a disease of chronic evolution whose nerve injury determines sensory and motor changes that lead to the installation of varying degrees of disability, especially in its most severe presentation: a lepromatous leprosy. Among severe disabilities and socially relevant, are skin ulcers. Sixty-eight percent (68 %) of the ulcers are located in the lower limbs (calcaneus, hallux, plantar and leg region) due to biomechanical alterations and decreased sensitivity developed in the patient. The biomechanical change occurs from amyotrophy, muscle weakness and deformities that contribute directly to the foot bone breakdown. This breakdown causes the patient perform a dysfunctional gait and cause new pressure points in regions not suitable foot. In addition, the patient with leprosy has decreased or abolished sensitivity, which reflects the reduction of physiological protection needed for the prevention of various skin lesions. Although less frequent, one should not ignore the occurrence of ulcers in other regions, including hands and therefore direct attention and focus of prevention for the same, which also represent risks to the patient (Gomes, Frade, Foss, 2007).

A variety of factors may predispose to malignant transformation of a chronic injury, such as long-term injuries, constant irritation, chronic infection, improper hygiene, environmental factors and genetic predisposition (Zagne Bauk, 2006). The development of squamous cell carcinoma (SCC) in chronic ulceration is a relatively unusual event (Wainstein *et all*., 2012).

The SCC is an atypical squamous proliferation of skin cells with invasive and metastatic potential character. Represents approximately 15 % of malignant neoplasms of the skin. It is the second most common type of cancer in people with light skin and more common in dark-skinned people. May occur in normal skin, although most often originates in cutaneous lesions prior, such as scars and chronic ulcers. Histologically malignant epithelial cells extending deeply toward the dermis as masses or cords. In low-grade squamous cell carcinoma, malignancy can be relatively well differentiated, resembling the mature squamous cells that can produce keratin and typical pearls corneas. In high-grade lesions, these epithelial cells can be extremely atypical, with figures of abnormal mitoses and lack of keratinization. As it is malignancy, early diagnosis is of paramount importance to ensure the best treatment conditions and prospects for cure (Wainstein *et all*., 2012). To establish it, the physician should consider the clinical characteristics of the lesions and primarily the result of the histopathological examination of the injured tissue (Barella, Blanco, Yamane, 2013).

This case is a typical example of Marjolin's ulcer, expression used to designate a malignancy, especially squamous cell carcinoma, occurring on chronic ulcers, fistulas or scars. Clinical findings that suggest malignancy include non-healing ulcers, increased consistency of the lesion, vegetation, unpleasant odor, raised edges and lumps on the wound. When a chronic ulcerated lesion undergoes modification in its clinical evolutionary aspect, becoming painful, infiltrated, hard, vegetative or secreting, the possibility of malignancy should be investigated by histopathology (Wainstein *et all*., 2012).

Overlapping disease is uncommon in medicine. However is not a rare event. This report demonstrates the importance of early diagnosis of leprosy, in order to prevent the development of neuropathic ulcers that can undergo malignant transformation. The squamous cell carcinoma in chronic ulcers of leprosy patients should not be thought as a rare occurrence. Moreover, early diagnosis by physicians should consider the possibility of malignant ulcers. Chronic ulcer must be monitored for the presence of a minimum of changes. Serial skin biopsies should be performed when any clinical lesion undergo changes.

Conclusion

The authors consider lentigo maligna melanoma occasionally found; showing no relation to leprosy, unlike the SCC seems to have a direct relationship.

For Medicine, Hansen's disease is no longer an obstacle. However, this disease remains highly prevalent in global statistics, constituting a public health problem. Early diagnosis is extremely important, not only for the prevention of its complications, as well as for the ultimate cure.

The lesions caused by leprosy should receive special care and attention, considering the possibility of malignant degeneration of injured long term, complicating the picture and often hindering accurate diagnosis. In this sense, the prevention of this complication should be segment of government actions in the fight against leprosy.

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Taguchi experimental design, a new method for prioritizing related factors in shaping the quality of hospital services (Case Study: Yazd Mojibian Private Hospital)

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Abstract

Introduction: In recent years, the rise of private hospitals has led to the considering customer satisfaction as the most important weapon in the competitive campaign. Due to this, hospital managers need to assess and improve the quality of services by using new tools to get ahead of their competitors. Concurrent use of the Fuzzy analytic hierarchy process (FAHP) and Taguchi experimental design techniques, presents a new approach that will enable organizations to form the most important factors that will improve the hospital services in non-linear way.

Materials and Methods: In this paper, we use the Delphi technique to identify the factors that constitute the consent of the patient. Then with regard to the dimensions of the SERVQUAL model, questionnaire was designed and distributed among 56 patients. For the analysis of the questionnaire data, the feature of multi-response Taguchi method was used.

Results: "Main effects" and "variance analysis" Tables showed that the most effective factors in improving the quality of hospital services and increase patient satisfaction, is "speed of work tending to patients' with participating share of 42.94 percent

Conclusion: In present study, the Taguchi experimental design method is applied to the field of manufacturing, service industry will also benefit from this approach. The approach proposed in this study can be used simultaneously to increase customer satisfaction and improve the quality of the product is associated with multiple criteria.

Key words: service quality, customer satisfaction, Hospital services, FAHP, Taguchi experimental design

Introduction

Nowadays the customer's satisfaction is the most important factor in organizations development and growth, and to get that goal we need improving services (Ting, 2004). When we are talking about services we should regard quality as one of strategic tools and it has different definitions (Ozdemir& Hewett, 2010).

Most researchers define quality of services as a general evaluation of services and many of them also regard it as a multi-dimensional structure which is constructed out of evaluating service characteristic(Mohammad Anber & Alhamadani, 2011). In fact, researchers regard the quality of services as presenting services above what is expected by customers (ForoughiAbari et.al, 2011). When we are talking about organizations which give services, we must regard hospitals as one of the most important categories which have an important role to enhance the society health and hygiene and if there is any fault, there will be strong outcomes (Boyer et.al, 2012). So having qualified personnel and having high quality services in preparing healthy accommodation for society will enhance the hygienic outputs and it will be in accordance to the modern professional knowledge (Akter et.al, 2013).

We should consider that there is no guarantee that the ideal level of preparing services in present time be acceptable in the future too. Therefore hospitals must have continious evaluation and then enhance their quality of services; also they should increase patient's satisfaction by developing new strategies (Siddiqi, 2011).

When a customer finds the services with highest quality, he/she will return to organization again

and again. Also if managers want to internalize two main concepts of efficiency and effectiveness in order toenhance the performance rate in organizations, they must upgrade the quality and focuson the tightened relations between factors of service quality and also understand the relationship between the quality of services and the customer satisfaction. Customer satisfaction in psychological context means, customer decide when he/she is finding his/her suppose services. Various researches have showed that there is a correlation between the quality of services and the customer satisfaction. So that enhancing the quality of services will result in customer satisfaction (Kuo et. al, 2011).

Customer satisfaction is a tool by which we can evaluate the organizations activities and it is based on experience got by the customers out of the services which organizations present those (Gupta & Gupta, 2012). When managers want to have an effective role they should regard quality as the most important factor in getting customers satisfaction and organizations success (Woodruff, 1997). It is clear that all organizations want to have the customer satisfaction so to get this goal, they must explore their customer needs and when they want to design and prepares their good and services they should maintain those needs (Garcia & Segura, 2009). If their services have an expected level of quality from the customer point of view, they will preserve their position and they will hold their customers in the best way.

In recent years, the importance of quality services in hospital has gained concentration from many researchers and they have done vast studies in this area. SoBuyukozkan and Cifci tried to give a combined approach of the Fuzzy Analytic Hierarchy process and Fuzzy Topsis on the quality of electronic services in hospitals. The results showed that hospitals must pay more attention to presenting special, mutual and accurate services and also there should be a system which answers to patient questions in order to enhance their satisfaction about the quality of services (Buyukozkan&Cifci, 2012). Muhammadbutt and Cyril also use Servqual tool to evaluate quality of services in private remedial centers in Malaysia. In order to do that, they selected 340 patients randomly and gave them Servqual questionnaires. The results showed that there is a negative gap in quality of remedial centers which can be analyze in five different aspects according to Servqual quality model (sympathy, guarantee, tangibility, responsibility and reliance) (Muhammad butt & Cyril, 2009).

Hatice and his colleagues try to show how we can use tool of enhancing quality to convert customer needs in to qualifiers of the service and enhancing the quality of services. In order to do so they used a compound approach of Servqual model and quality function deployment model in hospitals, and made it clear that how the personnel behave about customers and how they think about them have the most important effect on enhancing quality levels of services in hospitals (Hatice et.al, 2013). So far, different models in evaluating the quality of services have been used among which the most important are Servqual model (Siddiqui&Shandkar, 2007), Kano model (Sauerwein et.al, 1996), Customer Relationship Management (Jagdish, 2003), (Johnson &Gustafsson, 2000), (Pai&Tu, 2011) and so on.Consider that although the mentioned models are used vastly when we are talking about quality services, analyzing criteria are mostly used in linear way. Today changes have encouragedresearchers to weigh criteria with regard to mental judgment and used nonlinear methods (Chen & Chuang, 2008). Therefore to do this, present study introduces the compound approach of Analytic Hierarchy process and Taguchi experimental design method too. Also by reviewing former studies on quality of services we can understand that in most of these studies the researchers use common methods like Servqual model, quality function deployment and so on, to evaluate, survey and enhancing quality of services. Oppose to other methods which have used by researchers so far, the approach of Taguchi experiments has a better average in quality factors and at the same time it reduces quality dispersal. Moreover by changing controllable input factors, effecting on the process, we can evaluate its effect on the output and the final products and we can detect key variables which have influence on the quality characteristic on the product (Taguch, 1987).

Regarding organizational changes in recent years and also considering the importance of industrial services, this study has used Servqual models available factors to combined Fuzzy Analytic Hierarchy process and Taguchi experimental design, also by using patients viewpoints it will

propose a suitable compound of criteria by which we can analyze the quality services in hospital, and finally we can help managers to enhance patients satisfaction.

Methodology

This study is practical regarding its goal, is descriptive regarding the rate of controlling variables and is a field and library research regarding the method of gaining data. Statistical population in this study, includes patients of Mojibian Hospital of Yazd, and we have used relation (1) by serving on 43 people in which, P=q=0.5, and %95 confidence level.

$$n= Z^2pq/d^2 \quad \text{..... (1)}$$

Research framework

In general, we regard three stages for doing this study, determinant weigh of servqual dimensions, experimental design and preparing the questionnaire and finally analyzing the data. These processes are shown in figure (1).

Taguchi experimental design

All of the processes have input, output, controllable and uncontrollable factors which affect the process of converting input to output. So process will be controllable if the degree of influencing is clear. Designing the experiments is the technique by which we can find the degree of influencing of each factor on process. Our goal is reducing the number of experiments, saving time and money, omitting unnecessary factors, indicating variables which have most influence on the process, determining importance of each variable and finding ideal conditions (Taguchi, 1987). Taguchi method is suitable for designing ideal structures even with considering the interactions between variables. By this method we can develop factorial experiments and analyses the results (Hong, 2012).

Fuzzy Analytic Hierarchy process

When somebody wants make a decision, he should oppose different criteria. In this situation he should use prominent methods in this field. AHP is one of the most famous methods by which we can make decisions. It is invented by Tomas Saaty in 1980, we can use this method when we have some competitive choices and some decision making criteria. The base of AHP method is on coupled comparison of choices and decision making criteria. To do this comparison we should gather data from decision makers and this will prepare decision makers to focus on two choices or criteria without any regard to external irritation (Saaty, 1980). But traditional AHP cannot reflect process in accurate way, special when problems are not defines pro-

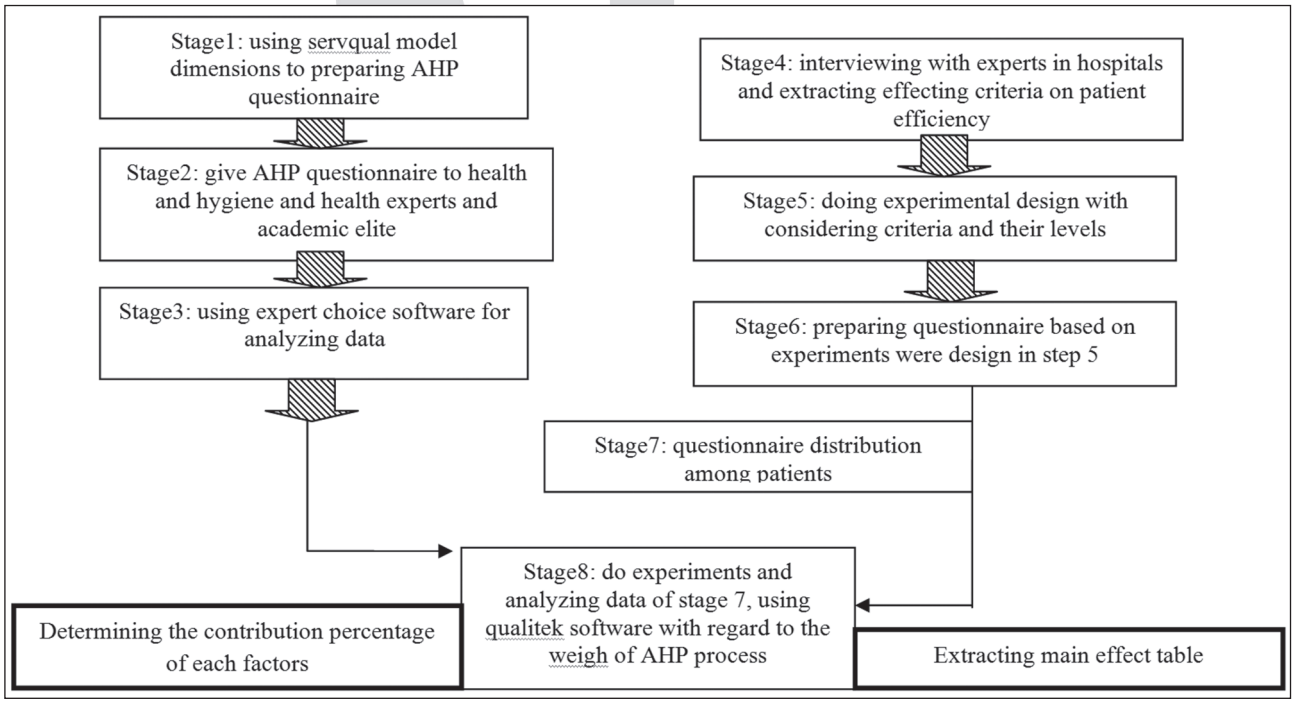

Figure 1. Research framework

perly also, Chang presented Extend Analysis Method that is more practical and easier than other methods (Saaty, 1989). In this study we have use, EA method so at first we evaluate the mathematical average of experts viewpoints to final matrix about coupled comparison, then we try to find S_k for each matrix lines of couples comparisons which is itself a triangle fuzzy number regard relation (2).

$$S_k = \sum_{j=1}^{n} m_{gi}^{j} \otimes \left[\sum_{i=1}^{n} \sum_{j=1}^{m} m_{gi}^{j}\right] \quad \text{..... (2)}$$

In which k, indicates line number and I and j indicates choices and indicators respectively. In EA method, we should find the magnitude rate of scales in relation with each other. Magnitude rateV (M1≥M2) is according to relation (3):

$$V(M_1 \geq M_2) = Sup\left[\min\left(\mu_{m_1}(x), \mu_{m_2}(y)\right)\right] \quad \text{.... (3)}$$

And also we have,

$$d(A_i) = \begin{cases} 1 \quad if m_2 \geq m_1 \\ 0 \quad if l_1 \geq u_2 \\ \dfrac{l_1 - u_2}{(m_2 - u_2) - (m_1 - l_1)} \quad otherwise \end{cases}$$

Magnitude rate of triangular number from k triangular fuzzy number is found from relation (4),

$$V(M \geq M_1, M_2, \ldots \ldots, M_k) =$$

$$= V[(M \geq M_1) and (M \geq M_2) and \ldots \ldots (M \geq M_k)] =$$

$$= \min V(M \geq M_i) i = 1,2, \ldots., K \quad \text{..... (4)}$$

Suppose that:

$$k = 1,2,3, \ldots., nd \;\; and \; (A_i) = \min V(S_i \geq S_k)$$

$$k \neq i,$$

Then weigh vector is as relation (5)

$$W' = (d'(A_i), d'(A_2, \ldots, d'(A_n))^T \quad \text{..... (5)}$$

$$A_i (i = 1,2,3, \ldots, n)$$

To normalize, normalized weigh vector are as follow:

$$W = (d(A_i), d(A_2, \ldots, d(A_n)))^T \quad \text{..... (6)}$$

Table (1), shows the scale by which we can convert exact numbers to triangular numbers:

Table 1. Relative importance of interval-valued fuzzy numbers

Assessment scale	Interval-valued fuzzy numbers of relative importance
Unimportant	(0, 0, 2)
Slightly important	(1, 2.5, 4)
Moderately important	(3, 5, 7)
Very important	(6, 7.5, 9)
Critical	(8, 10, 10)

Servqual model

Servqual is a practical framework to understand service qualities which were introduce by Parasuraman and his colleagues in 1995. Servqual means quality of services and it is a model by which we can enhance the quality of service by what customers want and what they expect (Naik et.al, 2010). Parasuran, Zeithmel and Berry, had studies on quality services and they proposed five dimension for quality service (Parasuraman et.al, 1985).

Determining the way of dimensions of servqual model

In this study we have used AHP to determine weigh of the model dimensions. In order to do so, we designed a questionnaire and asked 18 experts in remedial services to give their point of views about the importance of each dimension based on coupled comparison. The Result is as shown below.

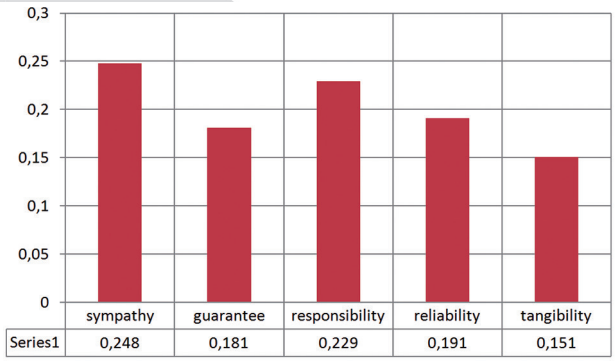

Figure 2. Results of AHP

Defining of experiments and preparing a questionnaire

First we had interviews with elite of remedial centers and hospitals and after deleting repetitive factors, we found 7 factors as the most important

Table 2. Dimensions of quality services (Parasuraman et.al, 1985)

Sympathy	Guarantee	Responsibility	Reliability	Tangibility
Paying attention to each of customers	Making customer trustful	Making the customers aware of timing	Doing tasks on time	Modern equipment
Doing job in appropriate time	Preparing safety and calmness	Preparing emergency services	Being interested in solving customer problems	Attractive physical accommodations
Having personal sympathy	Having respect to customer	Inclination for giving services	Doing something without need repetition	Neath appearance of employees
Understanding special needs of customers	Having enough knowledge to answer to customers	Spending time to answer customer questions	Making faultless record	

Table 3. Experiments design result

Number of experiment	Speed of solving patients problems	Being precise in process of giving services	Having respect and good behavior	Cleanness and attractiveness of personnel	Physical appearance of the equipment	Obeying law by employees	Being Patient in answering patients
1	weak	weak	weak	weak	weak	weak	weak
2	weak	average	average	average	average	average	average
3	weak	good	good	good	good	good	good
.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....
18	good	good	average	weak	average	good	good

1. Speed of solving patients problems by doctors and nurses
2. Being precise in process of giving services to patients
3. Having respect and good behavior with patients
4. Cleanness and attractiveness of personnel and their uniform overall
5. Physical appearance of the equpment
6. Obeying law by employees
7. Being Patient in answering patients

Considering three situations, weak, average and good, for each factor, we regarded 7 factors which are design in three levels and by using orthogonal arrays we designed 18 experiments. The results are as shown in table (3).

To make the questionnaire, we utilize results of different experiment and asking patient that: What was their sensation if they were in each 18 situations.

Data analyzing

In this stage, after gathering data, which are taken out of the questionnaire we used taguchi analyzing method to indicate the share of participation for each factor of the quality services in hospitals according to elite viewpoint and also finding the most effective factor in getting patient satisfaction. We can say that usually different methods of optimizing the experiments, regard one factor and the goal factor but, since in this study we have several factors as goal factors, we must compound these goals with each other, in order to do so, and regarding the abilities of qualitek software and also due to servqual model criteria weights which regard to AHP method, we used patient viewpoints and those before mentioned weights to analyze our data.

In this study, we used OEC method to change different factors to one factor. And also we used FAHP to give those factors special importance. The value of OEC can be obtained by using equation (7).

$$OEC = \sum Wi[yi - m]/[yb - ys]$$

In this relation, the "y" is the numeric value obtained from each experiment and in each separated experiments out of the general experiments and "yb" and "yf" are the largest and smallest values respectively regarding each experiment out of general. Also "m" is unwanted value out of each experiments and "*Wi*" is the percentage of its importance regarding each of the answers valued out of the general experiments. It should be mentioned that this equation is responding better if we regard responding calculations regarding the fact that if those calculations be done more we will find better answer (Taguchi, 1987)

Main effects of each factor

Main effect of factor in level L equals the total of response in level Ldevided by the number of response (Taguchi, 1987). And the results are shown in table (4).

We can say that, the more the mere value (lmax-lmin), the more is effect on the evaluated quality. Regarding the data on table (4), the mere value (lmax-lmin) for factor of speed of answering is more than other factors so this factor has the most effect on service quality and customer satisfaction. Moreover regarding that the more response is the better, the suitable level of each factor is the level in which the main factors effect is more. As we can see, the suitable level of all available factors at the above table is level 3, and we can declare that all patients expect all factors in a good level so that they will have the most satisfaction. In this section, diagrams 1-7 show the factors effects.

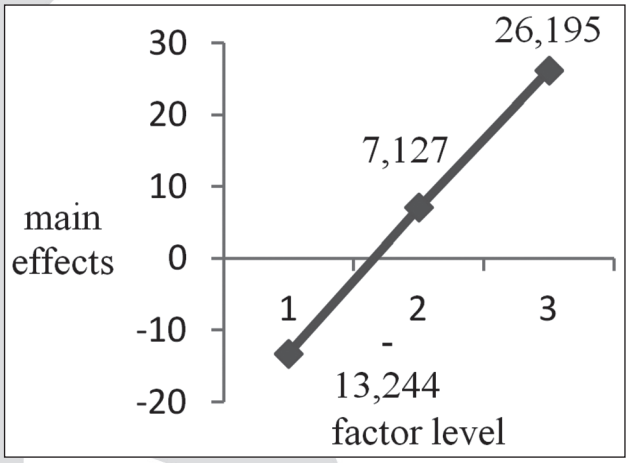

Diagram 1. Main effects for being patient in answering factor

Table 4. Main effects of each factor

Factor	Level 1	Level 2	Level 3	L_{MAX}-L_{MIN}
Speed of solving patients problems	-37.17	25.87	31.37	68.54
Being precise in process of giving services	-11.69	4.525	27.24	38.93
Having respect and good behavior	3.916	4.946	11.215	7.299
Cleanness and attractiveness of personnel and their uniform overall	4.297	6.795	8.986	4.689
Physical appearance of the equipment	5.331	-9.136	23.882	18.551
Obeying law by employees	0.62	-8.271	27.728	27.108
Being Patient in answering patients	-13.244	7.127	26.195	39.439

Table 5. Variance analysis

Factor	DOF (f)	Sum of Sqrs. (S)	Variance (V)	F_Ratio (F)	Pure Sum (S')	Contribution share P (%)
Speed of solving patients problems	2	17409.269	8704.634	10.913	15814.13	42.94
Being precise in process of giving services	2	4590.723	2295.361	2.887	2995.583	8.135
Having respect and good behavior	2	187.279	93.639	0.117	·	·
Cleanness and attractiveness of personnel and their uniform overall	2	66.057	33.028	0.041	·	·
Physical appearance of the equipment	2	3287.179	1643.589	2.06	1692.039	4.595
Obeying law by employees	2	5219.482	2109.741	2.645	2624.342	7.127
Being Patient in answering patients	2	4668.022	2334.011	2.926	3072.883	8.345
Other/Error	2	2392.709	795.569			28.85%
total	17	36820.724				100.00%

Determining participation share for each factors

Taguchi experiments have very valuable results, for example we can find how much each factor is effective in Dispersion of responses. In fact the contribution percentage for each factor, is determined by the share participating of that factor and calculating it is done by variance analysis (see table 5).

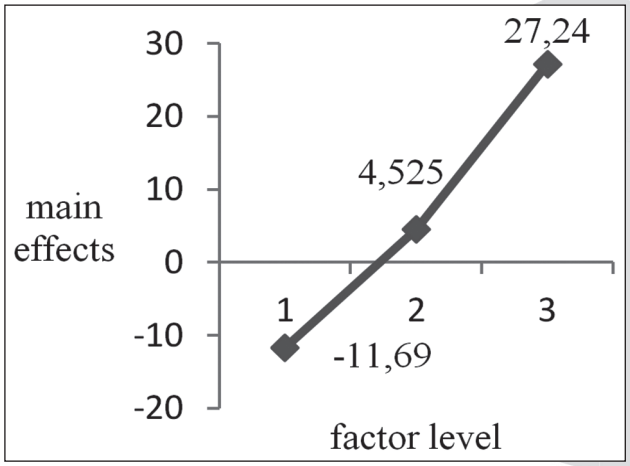

Diagram 2. Main effects of being precise factor

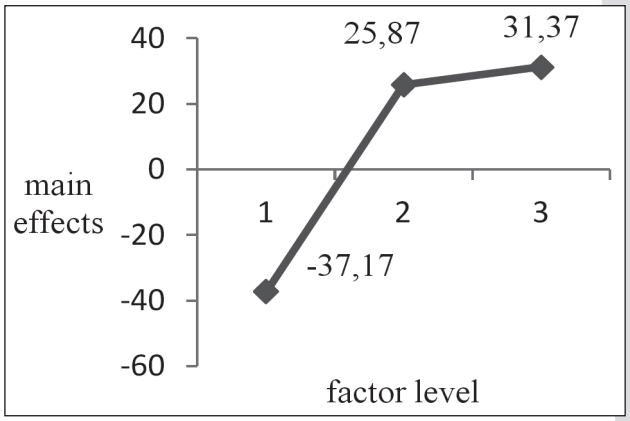

Diagram 3. Main effects of speed of solving problems

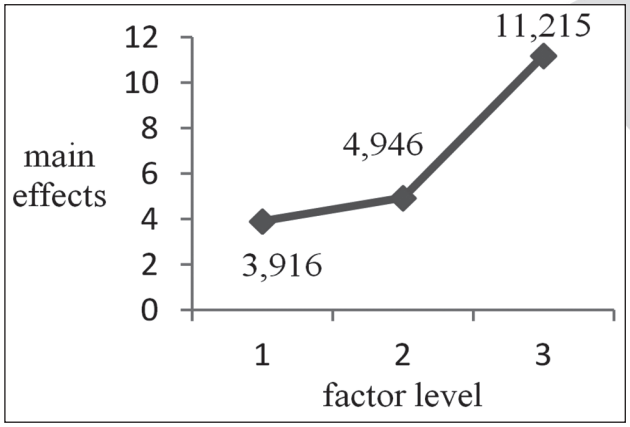

Diagram 4. Main effects of having respect

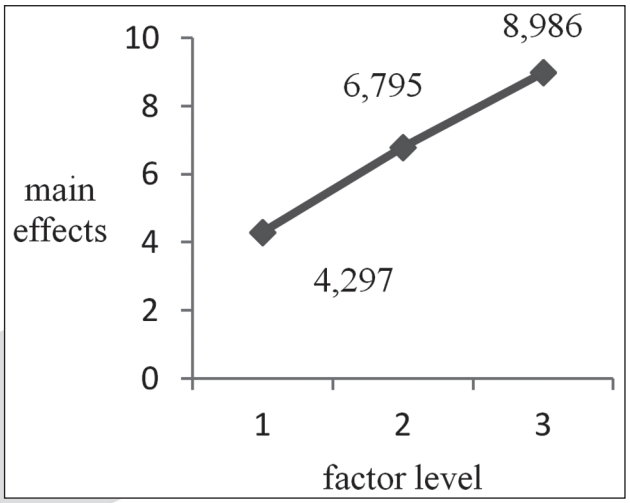

Diagram 5. Main effects of cleanness of personnel

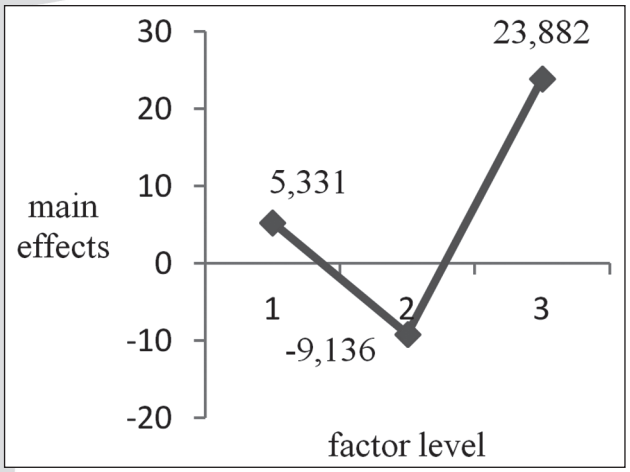

Diagram6. Main effects of physical equipment

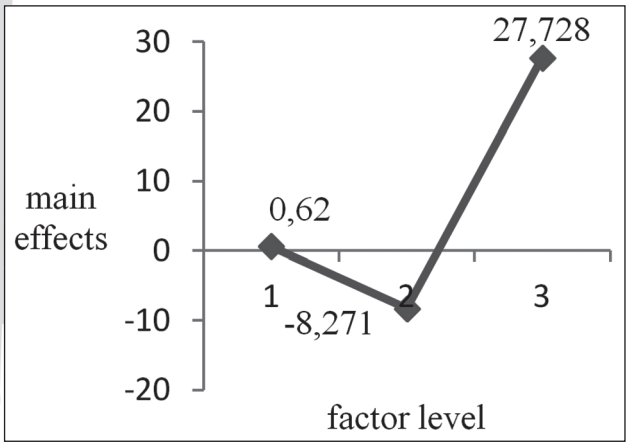

Diagram 7. Main effects of obeying law by employee

Regarding the above table, speed of solving patient problems has 42.94 % contribution share, and it has the most important effect on service quality in hospitals and. Also patients in answering the patients, accurate wok by the employees,

obeying law by employees and physical appearance of equipment have %8/345, %8/135, %7/127 and %4/595 contribution share respectively

Discussion

Nowadays, servicing organization is the most important factor when we are talking about economical growth in society. Remedial sections also have an important role in this growth (Pakdil& Harwood, 2005). In the modern era, the growth of private remedial centers has had a competitive role in attracting patients. Hospitals try to prepare suitable services and get patients satisfaction, regarding this fact in this study we have tried to explored and classified different factors that are related to patient satisfaction and we have used a compound approach which is prepared by compounding FAHP and Taguchi experimental design and finally by constructing a proper framework out of multiple criteria we have tried to show how much patient satisfaction will grow whenever the hospital services are growing. To do so, first we tried to ask remedial elites viewpoints and then by using different criteria which have introduced in Servqual model and using extracted results out of questionnaires, finally o analyze results, we used Taguchi method and also weight of Servqual model were calculated by using AHP regarding another necessary input needed by Taguchi method.

Conclusion

Regarding the table of main effect and variance analyzing, the results showed that the most important factors in improving hospital service quality and enhancing patient satisfaction, is speed of solving patient problems with 42.94 % contribution share, also patients in answering the patients, accurate wok by the employees, obeying law by employees and physical appearance of equipment have %8/345, %8/135, %7/127 and %4/595 contribution share respectively. The approach which is proposed in this study can be use simultaneously for enhancing the patient satisfaction and also enhancing the quality of service. We propose some items to get better results: Using available technique to evaluating work and also regarding the time and then finding exact time needed for each service so that the hospital managers can detect those works which consumes more time and also evaluate the personnel work skills and knowledge. Also hospital can use modern technologies to do so. Providing self-service facilities when people inter to hospitals so that a vast range of patients need are consider quality, another suitable strategy is holding workshops for employees who are in direct contact with patients. Another solution is making processes as simple is possible, making a balance between the volume of works and the patients needs on different shift, reducing the replacement of the personnel and encourage the ideal personnel force.

For future studies we recommend the qualified researches to use QFD model and find suitable solution of strategies regarding the above mentioned preferences. Also its desirable to do this study in Fuzzy or Grey situation and its result be compared with the results of this study.

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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
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Right margin	18 mm
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Regular paper may be divided in a number of sections. Section titles (including references and acknowledgement) 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)

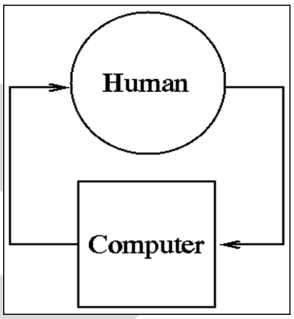

Figure 1. Text here

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.

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