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A comparison of dietary intake with Recommended Dietary Allowances (RDA) for colorectal cancer patient before and after different modulates of treatment

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Abstract

Background: Diet has been identified as a major determinant of colorectal cancer (CRC) but little is known of its influence on CRC survival. Diet is regarded as one of the most important environmental factors associated with colorectal cancer (CRC) risk. A recent report comprehensively concluded that total energy intake does not have a simple relationship with CRC risk, and that the data were inconsistent for carbohydrate, fat and protein.

The objectives of this study was to identify the dietary intakes of total energy, protein, fat, carbohydrate, fibre, and micronutrients and compare it with recommended dietary allowances (RDA) for colorectal cancer patients before and after different modulates of treatment (Surgery, Chemotherapy and Radiotherapy) in king Abdul-Aziz University Hospital (KAUH).

Subjects and methods: A cross- sectional descriptive study was carried out among (30) Patients (17) males (56.7%) while the other (13) females (43.3%) at King Abdul Aziz Hospital (KAUH). All the patients diagnosed with colorectal cancer during the months of November and April 2012. The data was collected by questionnaire include demographics characteristics and a 24-hr recall for 3 days before and after the treatment, laboratory investigations collected from the patient's medical records. Data of food intake were coded and analyzed using a computer program. Results were compared with RDA. The results were analyzed by SPSS statistical package version 15.

Results: Majority of patients (83.33%) have the tumor in the colon while only (16.66%) in the rectum. About (43.33%) treated with both Surgery and chemotherapy while (26.66%) surgery only, (13.33%) received only chemotherapy ;(13.33%) received a combination of Chemotherapy and Radiotherapy, and only (3.33%) treated with both Surgery and Radiotherapy. Total kilocalories, protein, fat, carbohydrates and fiber intake before treatment were 90.15%, 76.31%, 62.28%, 95.29% and 26.98% from RDA and after treatment increased to 111.25%, 95% , 83.97%, 108% and 20.57% from RDA. There was statistically significant differences ($P<0.001$, $P<0.01$, $P<0.05$, and $P<0.01$) for Kilocalories, protein, fat, and carbohydrates respectively. Generally the intake of water soluble vitamins and fat soluble vitamins after treatment for male and female patients were more than before with statistically significant differences intake between before and after treatment in (vita, A, D, B2, and Niacin in male patients) and for (vita, A, D, E, C, B1, B6 and Niacin in female patients).

The same results showed in minerals the intake increased after treatment for male patients than before treatment with statistically significant in (Calcium, sodium, phosphorus, potassium and Magnesium in male patients $P<0.001$,$P<0.01$, $P<0.05$, $P<0.01$and $P<0.001$ respectively). Also statistically significant differences intake between before and after treatment in (Calcium, iron, sodium, phosphorus, Zinc and Magnesium in female patients, $P<0.05$, $P<0.01$, $P<0.05$, $P<0.01$, $P<0.05$, $P<0.01$ respectively) for female patients.

Conclusion: Good nutrition practices can help cancer patients maintain weight and the body's nutrition stores, offering relief from nutrition impact symptoms and improving quality of life. Optimum nutritional care requires multidisciplinary

work within the framework of the protocol for therapeutic nutrition programmed.

Key words: Dietary intake, Recommended Dietary Allowances (RDA), Treatment Modules (Surgery, Chemotherapy and Radiotherapy).

Introduction

Colon cancer forms in the tissues of the colon (the longest part of the large intestine). Most colon cancers are adenocarcinoma which begins in cells that make and release mucus and other fluids[1]. Colorectal cancer (CRC) is the second most prevalent cancer and the third leading cause of cancer deaths worldwide, resulting in almost half a million deaths every year[2]. It is often diagnosed at a late stage, and those with metastatic disease have a 5 yr survival of approximately 7 %. The highest incidence rates occurred in North America, Australia, Western Europe and Japan. The incidence tends to be low in Asia and intermediate in the southern parts of South America. Although the kingdom of Saudi Arabia (KSA) is considered a low incidence area for CRC, the disease ranks second, after breast cancer [3]. There were 907 cases of colo-rectal cancer accounting for 9.9% of all newly diagnosed cases in year 2007 [4].

Geographic differences for CRC are probably explained by dietary and other environmental exposure[5]. Diet is regarded as one of the most important environmental factors associated with CRC risk[6, 7] particularly when diet is considered in the context of other energy balance indicators such as body size, physical activity, and alcohol or tobacco intake[8,9,10, 11]. It is especially important to eat as healthy a diet as possible to boost immune system and deal with possible nutritional deficiencies[12]. Protein helps the body repair and fight infection. Include a protein source with each meal or snack. Good sources of protein include lean poultry, meat, fish, eggs, low fat dairy products, nuts and nut butters, seeds, legumes, and tofu[13].

Eating less red meat and avoiding processed meat altogether can slash colon cancer risk, consuming less alcohol, boosting fiber intake, exercising, and maintaining a healthy body weight could prevent 45% of all colon cancer cases or more than 64,000 cases of colon cancer each year[14].

Carbohydrates provide the body with energy, vitamins, minerals, phytochemicals and fiber. Good sources include bread, grains, cereals, pasta, and rice. During treatment, if patient is able to tolerate high fiber foods, choose whole wheat bread, whole-wheat pasta, brown rice, oatmeal and quinoa. If patient cannot handle the fiber during treatment, consume refined grains such as white rice, ready-to-eat cereals, and enriched (white) bread [13].

Nutrition plays major (but not always fully understood) roles in many aspects of cancer development and treatment [15].

The three main types of treatment for colorectal cancer are Surgery, Radiation therapy and Chemotherapy. Depending on the stage of cancer, 2 or more types of treatment may be used at the same time, or used one after the other[16].

The purpose of this work was to identify the dietary intakes and compare it with recommended dietary allowances for colorectal cancer patients before and after different modulates of treatment (Surgery, Chemotherapy and Radiotherapy) in king Abdul-Aziz University Hospital (KAUH).

Subjects and methods

A cross- sectional descriptive study was carried out among (30) Patients at King Abdul Aziz Hospital (KAUH) with diagnosed colorectal cancer to be included in the study at their first visit to the outpatient surgery and oncology department between October 2011 and April 2012 were included in a retrospective review of the patients' medical record. Inclusion criteria for those Patients of both sexes aged over the age of 18 years old with colorectal cancer; proven adenocarcinoma of the colon involving any location from the cecum to the rectum; received any type of treatment. All racial and ethnic groups as well as both genders were included in this study. Of these, 13 (43.3%) female and 17 (56.7%) male were conducted. The mean age for both women and men was 51.96 ±1.0 2. Years (range 21-80 years). Patients with other diagnoses were excluded. The questionnaire contains several sections:

Socio-demographic data

This includes questions on basic socioeconomic characteristics of the households. It also collects data on individual characteristics as the: age, educational status of the patient and the wife / husband (if married), occupational and em-

ployment status, working hours, income source, average of household income, place of residence, and type of dwelling.

Medical history data

This is divided into two divisions: pre and post treatment. Questions in this section includes the location of the primary tumor, signs and symptoms, evidence of metastasis, area affected (if metastatic), family history of the disease, surgical history, medical history, medications, types of treatment, and finally the doses and sessions (if chemo or radiotherapy).

Daily intake of nutrients

In this study the researcher met with diagnosed colorectal Cancer patient to be included in the study at their first visit to the outpatient surgery and oncology Clinic and interviewed them to fill the questionnaire then they recorded the food intake by taking of the patient's 24-hr recall for 3 days before and after treatment.

Analysis of Food intake

The 24-hour recall method was used to assess the usual intake of energy and nutrients for three consecutive days. In the same day of interview, the patients were asked to recall type and quantity of all foods and beverages consumed during the previous 24 hours, and they were also asked to record the food intake during the another two days in their homes. Three-day-weighed food records were completed for all patients (n_30) participating in the study and assumed to be sufficient for estimating energy and protein intake on an individual basis. The intake of macronutrients, energy, protein, fat, and carbohydrates and micronutrients were analyzed for each patient using a computer program. The analysis by this program is based on food composition tables of the Egyptian National Nutrition Research Institute (ENNI) and on food composition tables for the Near East FAO 1982 [17]. Data of the 24-hour food intake were coded and entered into this program. The food intake data were analyzed using a computer program. Results were compared with current recommendations for nutrient intakes according to age and sex of participate (Male& Female). [18]

Ethical Considerations

Permission was attained from the head of department of Surgery and Oncology Clinic in King Abdul-Aziz university hospital (KAAUH).Patient was given consent before the interview.

Statistical analysis

Descriptive Statistics: arithmetic mean or average, median and standard deviation. Explore provides more descriptive statistics, including the standard errors. The results were analyzed by SPSS statistical package version 10 (1994)[19] and the results were tabulated by Harvard graphics packages version 4[20] were used for representing the results graphically.

Independent t-test was used to compare between the two sample means and F-test (One way ANOVA) were used for comparing between groups, there are two assumptions underlying the analysis of variance and corresponding F test. The first is that the variable is normally distributed. The second is that the standard deviation between individuals is the same in each group .If the F ratio is significant, and then SPSS conduct post hoc tests as LSD test (Least Significant Difference). A significant P-value was considered when P is less than 0.05 [21].

Results

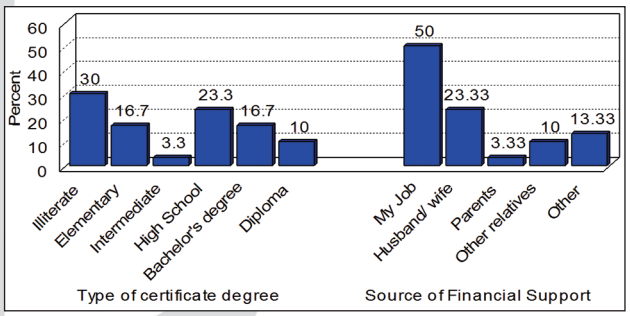

Figure 1. Percent distribution of type of certificate degree and source of financial of all samples

Figure 1 demonstrates percent of type certificate and source of financial support of the colorectal patients. As we can see 30 %, 16.7 %, 3.3 %, 23.3 %, 16.7 %, and 10 % were Illiterate, elementary degree, intermediate degree, high school degree, Bachelor's degree, and Diploma respectively. Regard to the financial support of the patients, 50%of them depend on their job, 23.33% from their husband / wife, only 3.3 % from their parents while 10 % from their relatives and 13.33 %from other sources.

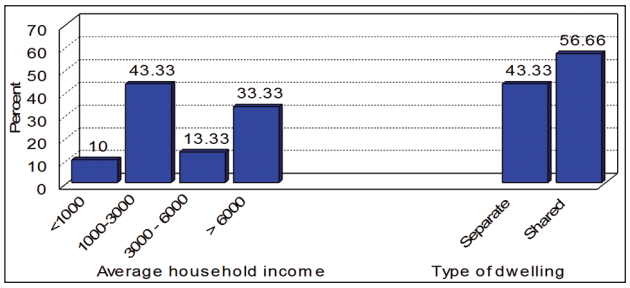

Figure 2. Percent distribution of the average household income and type of dwelling of all participated

Ten percent of patients were have a low income of less than 1000 Riyals per month, 43.33% of the participants have an income between 1000 to 3000 and 13.33 % between 2000 to 6000 Riyals, and finally 33.33% have more than 6000 Riyals per month. However the type of dwelling where 43.33 % of them live in a separate house and 56.66 % lives in a shared house as figure 2.

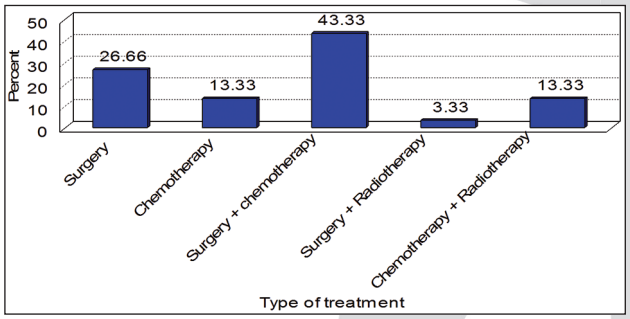

Figure 3. Percent distribution of type of treatment for all of colorectal cancer patients

Figure 3 shows the different types of cancer treatment including surgery, chemotherapy and radiation. The majority of the patients (43.33%) treated with both Surgery and chemotherapy while (26.66%) treated by surgery only, (13.33%) of the patients received only chemotherapy and other (13.33%) of them received a combination of chemotherapy and radiotherapy, and only (3.33%) treated with both surgery and radiotherapy.

The mean ± SD and % RDA of caloric and Macronutrients intake for male and female studied patients are represented in table (1).

For male patients: The daily dietary intake from protein and carbohydrate as percent from RDA before treatment were (76.31±2.97) and (95.29±4.67), respectively, and (95.39±4.93, 108.39±5.98) for after respectively and the difference was statistically high significantly (P< 0.01). However the daily dietary intake from fat as percent from RDA was (62.28±3.71) before and (83.97±4.03) for after and the difference was significant at (P< 0.05) also the daily dietary intake from fiber as percent from RDA before and after treatment was (26.98±0.89) and 20.57±1.9 respectively and there is no significant difference.

For female patients: The caloric intake as percent from RDA was (67.29±3.25) before which increased to (85.47±2.47) after treatment and the different were statistically significant (p<0.001). However daily intake of protein as percent of RDA before and after treatment was (53.37±1.88, 73.78±6.65)

Table 1. Mean ± SD and % RDA of Macronutrients intake for male and female studied sample of colorectal cancer patient before and after treatment

Variables	Before		After		T. value	P
	M±SD	%RDA	M±SD	%RDA		
Male						
Caloric(Kcal/d)	1857.92±5.59	90.15±2.02	2194.66±4.34	111.25±6.54	7.36	***
Protein(gm/d)	67.68±2.76	76.31±2.97	79.63±3.68	95.39±4.93	2.99	**
Fat (gm/d)	40.09±1.31	62.28±3.71	53.78±2.88	83.97±4.03	2.01	*
Carbohydrate (gm/d)	306.58±1.27	95.29±4.67	348.02±4.55	108.39±5.98	2.92	**
Fiber (gm/d)	6.14±0.32	26.98±0.89	4.72±0.11	20.57±1.9	1.18	NS
Female						
K Caloric (Kcal/d)	1684.91±8.27	67.29±3.25	2118.2±8.26	85.47±2.47	3.23	**
Protein(gm/d)	55.19±2.16	53.37±1.88	62.83±3.6	73.78±6.65	4.85	***
fat (gm/d)	39.4±1.67	70.38±2.71	43.59±2.56	77.82±4.48	1.4	**NS**
Carbohydrate(gm/d)	277.36±2.31	102.63±5.9	368.63±2.17	136.56±7.68	0.84	**NS**
Fiber (gm/d)	6.98±0.33	35.25±1.55	8.99±0.53	45.41±2.21	2.19	*

** P< 0.05 ** P< 0.01 *** P< 0.001 NS: No significant*

respectively which is statistically highly significant (p<0.0001), Regard to the daily dietary intake from fat as percent from RDA was (70.38±2.71) before and (77.82±4.48) for after but there's no significant and for the carbohydrates intake as a percent of RDA before and after treatment was (102.63±5.9, 136.56±7.68) respectively which demonstrate no significant differences. Also the fiber intake as a percent of RDA before and after treatment was (35.25±1.55, 45.41±2.21 respectively), this different was significant (p<0.5).

Table (2) shows the Mean± SD of the daily intake of minerals before and after treatment and the daily dietary intake as percent from RDA for the male and female of the colorectal cancer patients.

For male patients: The differences between before and after daily intake as percent from RDA for calcium, sodium and zinc demonstrate significant (P< 0.05). However the differences between before and after for Calcium and magnesium demonstrate statistical highly significant (p< 0.0001). Regard to the daily dietary intake of cholesterol after treatment was higher than before where the Mean± SD before treatment was 246.26±1.74 and after treatment was 296.83±3.07 but the different between before and after was statistically significant (p<0.001). Likewise daily intake from sodium and potassium increased after treatment than before and statistically significant increased (p<0.001).

For female patients: The Mean± SD intake as pe3rcent of RDA for the minerals and cholesterol intake before and after treatment for calcium, iron, sodium, phosphorus, potassium, zinc and magnesium before the treatment. The differences between before and after for calcium, sodium and zinc was significant (P< 0.05).However the differences between before and after for iron, Phosphorus and magnesium were highly significant at (p< 0.01) but for potassium there were no significant.

Table (3) shows the Mean± SD of the intake of vitamins before and after treatment for the male and female of the colorectal cancer patients and the daily dietary intake as percent from RDA. For male patients: The daily intake from water soluble vitamins: vit.C, folate, vit.B1, vit.B2, Niacin, vit.B6, and vit. B12 before treatment as percent from RDA were (147.75±2.93, 197.97±3.77, 71.56±3.87, 113.57±7.79, 80.4±2.9, 96.32±6.1 and 113.23±5.47 respectively). While after treatment the intakes of these vitamins as % from RDA

Table 2. Mean± SD and % RDA of minerals and Cholesterol intake for male and female studied sample of colorectal cancer patients before and after treatment

Variables	Before		After		T. value	P
	M±SD	%RDA	M±SD	%RDA		
Male						
Calcium (Mg/d)	635.25±3.48	141.16±7.21	838.06±6.81	186.23±3.73	5.56	***
iron (Mg/d)	11.87±0.41	197.84±3.51	12.36±0.61	206.07±5.06	1.62	NS
Sodium (Mg/d)	2100.97±8.23	136.22±6.41	2673.3±6.3	174.34±5.87	2.99	**
Phosphorus(Mg/d)	1008.6±8.21	144.08±6.8	1166.89±2.84	166.69±8.4	2.79	*
Potassium(Mg/d)	1828.98±8.11	389.14±6.4	2123.88±3.6	451.89±11.4	3.03	**
Zinc (Mg/d)	8.01±0.83	72.88±2.74	9.24±2.11	84.01±3.44	1.01	NS
Magnesium(Mg/d)	240.25±2.57	57.36±2.79	380.91±1.87	90.79±2.97	5.06	***
Cholesterol (Mg/d)	246.26±1.74	82.08±2.24	296.83±3.07	98.94±4.35	2.87	**
Female						
Calcium(Mg/d)	600.72±2.52	133.49±5.89	688.06±3.71	152.9±1.93	2.01	*
iron (Mg/d)	9.63±0.81	68.31±2.46	12.16±0.81	80.62±5.87	2.19	**
Sodium(Mg/d)	1917.46±2.93	144.44±6.32	2241.68±4.49	169.39±4.66	2.75	*
Phosphorus(Mg/d)	909.69±6.06	129.95±6.58	1068.58±6.63	152.65±6.66	3.36	**
Potassium(Mg/d)	1776.69±3.8	378.01±2.25	319.43±1.06	471.25±5.29	1.37	NS
Zinc (Mg/d)	7.59±0.28	94.9±5.61	9.53±0.63	119.13±5.17	2.23	*
Magnesium(Mg/d)	272.29±4.41	85.09±4.5	319.43±5.06	99.82±5.7	2.87	**
Cholesterol(Mg/d)	196.54±4.58	65.51±1.52	187.38±4.54	62.46±4.18	0.94	NS

** P< 0.05 ** P< 0.01 *** P< 0.001 NS: No significant*

Table 3. Mean± SD and % RDA of vitamins intake for male and female studied sample of colorectal cancer patients before and after treatment

Variables	Before		After		T. Value	P
	M±SD	%RDA	M±SD	%RDA		
Male						
Vit.A (Mcg/d)	623.75±3.6	83.16±5.01	974.13±1.88	129.88±1.91	2.6	***
Vit.C (gm/d)	44.32±3.87	147.75±2.93	47.01±1.57	156.68±3.58	0.98	NS
Folate(Mcg/d)	395.95±3.55	197.97±3.77	240.11±2.87	120.05±1.43	1.62	NS
Vit.D (Mg/d)	1.38±0.28	16.6±2.65	1.67±0.12	22.5±1.63	2.96	*
Vit.E(Mg/d)	13.03±2.43	86.9±2.87	14.42±1.3	96.15±4.25	0.33	NS
Vit.B1(Mg/d)	0.85±0.01	71.56±3.87	0.97±0.06	81.37±2.03	1.81	NS
Vit.B2(Mg/d)	1.47±0.12	113.57±7.79	1.7±0.01	130.76±7.35	3.81	*
Niacin(Mg/d)	12.86±1.1	80.4±2.9	16.29±1.91	101.83±6.94	3.47	**
Vit.B6(Mg/d)	1.45±0.54	96.32±6.1	1.49±0.07	100.23±5.09	1.16	NS
Vit.B12(Mcg/d)	2.71±0.29	113.23±5.47	2.84±0.15	118.38±7.49	2.17	NS
Female						
Vit.A(Mcg/d)	400.72±3.97	53.42±2.46	661.14±3.14	88.15±2.75	2.32	***
Vit.C(Mg/d)	62.05±2.38	206.84±7.93	106.33±5.76	354.46±2.55	3.8	***
Folate(Mcg/d)	210.93±8.3	105.46±5.15	274.85±2.19	137.42±6.09	1.18	NS
Vit.D(Mg/d)	0.89±0.07	14.53±1.54	1.3±0.07	23.46±1.73	3.03	*
Vit.E(Mg/d)	15.14±0.67	100.97±4.95	25.22±1.38	168.15±1.35	2.13	*
Vit.B1(Mg/d)	0.67±0.01	61.53±2.81	0.93±0.05	85.31±5.89	2.18	*
Vit.B2(Mg/d)	1.13±0.06	103.49±5.73	1.4±0.07	127.27±7.49	1.33	NS
Niacin (Mg/d)	11.53±0.76	82.36±4.31	14.76±1.52	105.43±7.93	2.06	*
Vit.B6 (Mg/d)	1.15±0.03	88.75±5.26	1.77±0.11	136.68±6.86	3.79	*
Vit.B12(Mcg/d)	1.76±0.12	73.39±4.81	1.93±0.16	80.76±4.62	1.53	NS

** P< 0.05 ** P< 0.01 *** P< 0.001 NS: No significant*

were (156.68±3.58, 120.05±1.43, 81.37±2.03, 130.76±7.35, 101.83±6.94, 100.23±5.09 and 118.38±7.49 respectively).

The mean ± SD of the daily dietary of fat soluble vitamins vit.A, vit. D and vit.E intake before treatment as percent from RDA were (129.88±1.91, 22.5±1.63 and 96.15±4.25 respectively). The differences between before and after for vit.A was highly significant (P< 0.001) and for niacin was highly significant (P< 0.01) while vit.D & vit.B2 was significant (P< 0.05) however there is no significant for the other vitamins.

For female patients: The same table illustrates the daily dietary intake as a percent of RDA before and after treatment for vitamin c was (206.84±7.93, 354.46±2.55 respectively) while the folate was (105.46±5.15, 137.42±6.09 respectively). The vitamin B1 was (61.53±2.81 before and 85.31±5.89 after), for vitamin B2 before was (103.49±5.73) and after was (127.27±7.49), the niacin intake before and after was (82.36±4.31 and 105.43±7.93 respectively), for the vitamin B6 was (88.75±5.26 and 136.68±6.86 respectively). Finally the intake as a percent of RDA before and after for the vitamin B12 was (73.39±4.81, 80.76±4.62 respectively).

Discussion

Colorectal cancer ranks the third highest in cancer incidence and fourth in cancer mortality in both sexes combined worldwide[22]. The role of dietary and other lifestyle factors in colorectal cancer recurrence and survival is largely unknown. Approximately 60% of individuals diagnosed with colorectal cancer will have surgery. In addition to these mechanical barriers, surgery frequently imposes an immediate metabolic response that increases the energy needs and changes the nutrient requirements necessary for wound hea-

ling and recovery at a time when baseline needs and requirements are often not being met [23].

The questionnaire was includes information about Socio-economic status demographic data including; educational level, income and a 24-hr recall for 3 days before and after the treatment.

The types of certificate degree were 30 %, 16.7 %, 3.3 %, 23.3 %, 16.7 % and10 % were Illiterate, elementary degree, intermediate degree, high school degree, Bachelor's degree and Diploma respectively as showed in figure (1). Our result revealed that about 33.3% of patient were had more than 6000 RS/ month, however 13.33 had 3000- 6000RS/ month and 43% were had 1000 to 3000 RS/month .About 57% from our participates were living in share dwelling but remain (43%) had separate house as seen in figure (2). This result verified that most of our sample in high Socioeconomic status (H-SES).

The different types of treatment

Nutrition is more than just food; it is an essential part of clinical care that can be improved. Many individuals also present with preexisting comorbid diseases and illnesses that further complicate their treatment. Surgery, chemotherapy, and radiation can have a direct (or mechanical) and/or an indirect (or metabolic) negative effect on nutritional status. The success of the anticancer therapy will be influenced by a patient's ability to tolerate therapy, which will, in turn, be affected by nutritional status preceding treatment[23]

Our result reported that the majority of the patients (43.33%) treated with both Surgery and chemotherapy while (26.66%) treated by surgery only, (13.33%) of the patients received only chemotherapy and other (13.33%) of them received a combination of chemotherapy and radiotherapy, and only (3.33%) treated with both surgery and radiotherapy as shown in figure (3). Many individuals experience fatigue, pain, and loss of appetite and are unable to consume their regular diet as the result of surgery[24]. Prompt nutritional therapy can help relieve or reduce these problems. Avoiding carbonated or known gas-producing foods will help, as will altering the fiber content in the diet to encourage bowel regularity. A well-balanced diet that contains the recommended amounts of essential nutrients and calories will help promote good wound healing. Finally, proper nutrition and adequate rest may help prevent or treat fatigue[24].

Surgery is often the primary treatment modality for cancer. Approximately 60% of individuals diagnosed with cancer will have some type of cancer-related surgery.[23]

Chemotherapy agents can be used in combination or as single agents, depending on the disease type and health condition of the individual[25]. Unlike surgery and radiation therapy, cancer chemotherapy is a systemic treatment (not a localized treatment) that affects the whole body (not just a specific part[26]. Consequently, there are potentially more side effects with chemotherapy than with surgery and radiation therapy [26]

Radiation Therapy Patients who maintain good nutrition are more likely to tolerate the side effects of treatment. Adequate calories and protein can help maintain patient strength and prevent body tissues from further catabolism. Individuals who do not consume adequate calories and protein use stored nutrients as an energy source, which leads to protein wasting and further weight loss. A prospective, randomized study of patients with colorectal cancer receiving radiation therapy demonstrated that concurrent individualized dietary counseling can improve patients' nutritional intake, status, and quality of life. These improvements, in turn, may reduce radiation-induced morbidity[27]

Baseline dietary intake

The 24-hour recall method was used to assess the usual intake of energy and nutrients for three consecutive days. In the same day of interview, the patients were asked to recall type and quantity of all foods and beverages consumed during the previous 24 hours, and they were also asked to record the food intake during the another two days in their homes. Three-day-weighed food records were completed for all patients (n_30) participating in the study and assumed to be sufficient for estimating energy and protein intake on an individual basis [28]. The Mean intake of macronutrients, energy, protein, fat, and carbohydrates and micronutrients were analyzed for each patient using a computer program. Results were compared with current recommendations for nutrient intakes according to age and sex of participate (Male& Female).

The mean daily intake of energy and nutrients at baseline are shown in table (1) which revealed that total kilocalories, protein, fat, carbohydrates and fi-

ber intake after treatment increased than before treatment in both male and female patients. National Nutritional council[28] report that a larger percentage of energy was derived from fat and sugar, and a smaller percentage from other carbohydrates than in the national recommendations. The daily intake of dietary fiber, vitamin D, ∝ - tocopherol, and magnesium was low, whereas the mean intake of other nutrients exceeded the national recommendations for healthy individuals[28]. High fiber was associated with an approximately 40% reduction in risk[29]. Gorham 2005[30] in conducted Observational study found that increased vitamin D intake is associated with a reduced risk of CRC. Calcium may also directly influence the proliferative activity of the colonic mucosa[29]. Norat and Riboli in 2003[31] conducted Observational studies have generally found that increased Calcium intake are associated with a reduced risk of CRC. The results of our study indicate that patients with CRC vitamins and minerals as percent from RDA increased after treatments than before treatments as shown in table (2&3).

Choban and Flancbaum, 2000[32] study indicates that patients with colorectal cancer generally were unaware of the importance of good nutritional status before surgery. Many of those with normal weight, as well as those with obesity, told the nurse they believed that losing weight before surgery was beneficial; however, unintentional weight loss in an obese patient can cause malnutrition and the same risk of complications as in a patient with a normal BMI.

Providing the patient with information about the importance of good nutrition enables the patient to improve his or her situation by self-care and thereby counteract malnutrition [32 & 33]. As emphasized by Ravasco. et, al (2005)[34], individual advice about nutrition reduces the frequency of complications for patients with rectal cancer undergoing radiation treatment.

Conclusion

Cancer Patients do have a real nutritional problem that surely can influence their disease course and length of hospital stay after surgery and long duration of receiving other treatment. Optimum nutritional care requires multidisciplinary work within the framework of the protocol for therapeutic nutrition programmed.

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Anxiety, depression and quality of life in patients with chronic hepatitis using Tenofovir and Lamivudine

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Abstract

Objectives: The study has been held as comparatively and descriptively with the aim of researching the side effects and effects to life quality, anxiety and depression level of Tenofovir (TFV) and Lemivudine use on patients with chronic hepatitis who have applied to the Sakarya Training Research Hospital (STRH) Infectious Disease Clinic.

Methods: 42 patients having been registered to STRH Infectious Disease Clinic using TFV and LAM have set the sample of the study. Data has been collected by using a questionnaire form consisting of socio-demographic qualities, SF-36 Life Quality Scale and Hospital Anxiety Depression (HAD) Scale. The data analysis has been held in computer environment by using t test, chi-square and ANOVA tests.

Results: 54.7 % of patients who have attented to the study are female and the average age is 44.83±12.37. It has been found out that 26.2% of the patients have anxiety, 40.5 % have depression; and the anxiety (16.7%) and depression (26.2%) possibility for LAM users is more than the ones using TFV; however there is no statistically significant difference between them (p>0.05).Examining the quality of life; the Physical Size point average has been found as 72.69±23.34 while the Mental Size point average is 70.16±23.26. In terms of the sub-dimensions of drugs and life quality; the only significant difference has been found in physical function (PF) sub-dimension (p=0.038) and it has been determined that the anxiety and depression are more common in patients using LAM.

Conclusions: There were no difference in terms of anxiety and depression and side effects of both drugs. But only, criterion that physical function was different in LAM group patients. This finding may be related with higher viral load in patients using TFV.

Key words: Tenofovir, Lamivudine, anxiety, depression, quality of life.

Introduction

Chronic hepatitis B and C are widespread all over the world. The lifetime risk of death due to liver diseases for men has been predicted as 40-50 % while it is 15% for women. For this reason, the chronic viral hepatitis (CVH) which is endemic among liver diseases is a serious health problem [1]. According to the data of World Health Organization; more than 2 billion people are infected with hepatitis B virus (HBV) and more than 350 million of them are chronic carriers [2,3]. About 17 million people are infected with hepatitis C virus (HCV) and every year 3-4 million people are getting infected with HCV [4].

Turkey is among the groups of countries having moderate endemicity in terms of Hepatitis B Infection [3,5]. According to the 2010 Department of Primary Health Care statistics; Hepatitis B incidence is found as 4.2/100.000 [6]. It is stated that the carrier rate of Hepatitis B is 2-8 % while chronic Hepatitis B (CHB) is 4-5 % [5]. However the Hepatitis B prevalence in Turkey also differs by regions. While there is 2-3 % positivism in the west side of Turkey, it is 7-8 % in east side and the HBsAg is higher in rural and poor communities [3].

The introduction of oral antiviral drugs in the treatment of chronic viral hepatitis has made significant progress and today some drugs like lamiduvin, adefovir dipivoksil, telbivudine, entecavir and tenofovir disoproxil fumarate have been involved in fumarate treatment options [7].

Antiviral treatment is a complex and long-term treatment depending on its compliance, continuity and side effects that can be greatly reduced [8]. During intiviral treatment, the most common side effects seen in more than 50 % of the patients are

post interferon flu-like symptoms, tiredness, headache, stomacache, nausea, diarrhea and laboratory abnormalities [9].

Lamivudin is one of hepatitis B reverse transcriptase inhibitors and is used in chronic hepatitis B virus infection [10]. In one the metaanalysis study, by seeing nonspecific gastrointestinal complaints, and minor side effects like erythema and paresthesia in only 2.1 % of the patients, the treatment has been interrupted [11]. When the effectiveness and reliability of tenofovirin is anaylzed, the most common side effects are stated as nausea, asthenia, prostration and headache [12].

It has been stated that while tenofovir is in affect, it leads to mitochondrial dysfunction by inhibiting human mitochondrial DNA polymerase and as a result even if it is so rare, there may be some side effects in patients using tenofovir as myopathy, neuropathy, lactic acidosis, macrocytasis, osteopenia and nephrotoxicity [9,12,13].

In the early stages of the disease; by leading to prostration, pyhsical symptoms like muscle and joint pain and psychological symptoms as depression; chronic viral hepatisis leads to impaired quality of life related with health due to some complications like cirrhosis, hepatocellular carcinoma, [14,15].

The quality of life and depression studies in patients having chronic viral hepatitis are generally held with the ones having IFN-a treatment in Turkey. For this reason, in this study we have aimed to find out the level of anxiety, depression and life quality in patients using lamivudine and tenofovir.

Material and Methods

While patients who are registered in STRH and using TFV and LAM constitute the universe of the study; 42 patients who has applied to Infectious Disease Clinic between 22.02.2011-26.10.2011 constitute the sample of the study. The study has been held after getting the permission from the institution and approval from ethics committee. Data has been collected by using a questionnaire form containing socio-demographic qualities, SF-36 Lide Quality Scale and Hospital Anxiety Depression (HAD) scale. The data analysis is made with SPSS program using t test, chi-square and ANOVA tests.

The Hospital Anxiety and Depression Scale (HAD)

It consists of 14 subjects in total -7 for depression and 7 for investigation of the anxiety symptoms- filled by the patient. The aim of the scale is not to get a diagnosis but to identify the risk group by scanning the anxiety and the depression in a short time in patients having somatic disorders. The HAD scale is developed by Zigmond and Snaith and the validity and reliability analysis has been completed [16]. In our country, the validity and reliability studies have been held by Aydemir (1997) and the break points for anxiety subscale (HAD-A) has been determined as 10, and 7 for depression subscale (HAD-D). The scoring for each subject is different in scale. 1., 3., 5., 6., 8., 10., 11. and 13. subjects show decreasing strength and the scoring is in the form of 3, 2, 1, 0. On the other hand, the 2., 4., 7., 9., 12. ve 14. subjects are scored in the form of 0, 1, 2, 3. While the subjects of 1, 3, 5, 7, 9, 11 and 13 are being put together for the anxiety subscale; for depression subscale the points of 2, 4, 6, 8, 10, 12 and 14 are being totalised. The lowest score that patients can get from both subscales is 0 and the highest one is 21. HAD is preferred for the reason of not having any subjects in relation to physical symptoms [17]. When the internal consistency of HAD scale is analyzed in our study; the alpha values of Cronbach is determined as 0.87 for HAD-A and 0.71 for HAD-D.

The World Health Organization Quality of Life Brief Form (WHOQOL-BREF): Brief Form 36 (SF 36) (Short Form)

It is developed by Ware in 1989 with the aim of evaluating the quality of life and regulated to be used in clinical practices and researches, evaluation of health policies and examination of general population [18]. It has been translated into Turkish and the validity and reliability studies have been completed [19,20]. SF-36 which has 36 statements consists of two main dimensions as physical and mental and 8 sub dimensions as social limitations due to physical function, social function and physical problems; role limitations due to emotional problems, mental health, energy (vitality), pain and general health perception [18,19].

Scores of living quarters scale related with the health varies between 0 and 100 from the lowest

to the highest [18,21,22]. Within this study, the internal consistency of the SF-36 has been analyzed again and the alpha values of Cronbach have been determined as 0.93 for physical dimension and 0.89 for mental dimension.

Results

54.7 % of the patients participating to the study are women, 90.5% of them are married, 52.4% are primary school graduates, 73.9% are middle income ones, 54.7% live in town and the average of age is 44.83±12.37. There is no significant difference between two groups having socio-demographic attributes and the groups have a homogeneous distribution (p>0) (Table 1).

Analyzing the side effects of the drugs; it has been seen that the most part of them have asthenia (40.5%), muscle pain - headache (28.6 %) and chills-shivering (19.0%); and there is no significant difference between two drugs in terms of side effects (p>0.05) (Table 2).

26.2% anxiety and 40.5% depression have been detected in patients. It has been determined that the ones using LAM have more possibility of having anxiety (16.7%) and depression (26.2%) compared with the ones using TFV; however there is no significant difference between them (p>0.05) (Table 3).

Table 1. Socio-demographic attributes according to the use of Tenofovir ve Lamivudine (N=42)

Socio-demographic attributes	Tenofovir n (%)*	Lamivudine n (%)*	X^2	p
Gender				
Female	8 (19.0)	15 (35.7)	0.019	0.890
Male	7 (16.7)	12 (28.6)		
Marital Status				
Married	12 (28.6)	26 (61.9)	2.972	0.122
Single	3 (7.1)	1 (2.4)		
Place of Residence				
Town	9 (21.4)	14 (33.3)	0.258	0.611
Downtown	6 (14.3)	13 (31.0)		
Job Status				
Employed	6 (14.3)	9 (21.4)	0.187	0.666
Unemployed	9 (21.4	18 (42.9)		
Income Status				
Low	2 (4.8)	9 (21.4)	1.995	0.273
High	13 (31.0)	18 (42.9)		

**The percentage in N has been estimated*

Table 2. Side effects according to the use of Tenofovir and Lamivudine (N=42)

Side effects	Tenofovir n (%)*	Lamivudine n (%)*	X^2	p
Fever	3 (7.1)	3 (7.1)	0.0062	0.649
Chills-Shivering	3 (7.1)	5 (11.9)	0.014	1.000
Muscle Pain-Headache	5 (11.9)	7 (16.7)	0.259	0.726
Asthenia	5 (11.9)	12 (28.6)	0.494	0.482
Anorexia	4 (9.5)	3 (7.1)	1.680	0.225
Nausea-Throwing up	3 (7.1)	4 (9.5)	0.187	0.686
Dermatitis	3 (7.1)	2 (4.8)	1.458	0.329
Alopecia	1 (2.4)	6 (14.3)	1.680	0.390
Concentration Disorder	1 (2.4)	3 (7.1)	0.221	1.000

** The percentage in N has been estimated*

Table 3. The relation of Tenofovir and Lamivudine according to anxiety and depression (N=42)

	Tenofovir n (%)	Lamivudine n (%)	X^2	p
Anxiety				
Yes	4 (9.5)	7 (16.7)	0.003	1.000
No	11 (26.2)	20 (47.6)		
Depression				
Yes	6 (14.3)	11 (26.2)	0.002	0.963
No	9 (21.4)	16 (38.1)		

** The percentage in N has been estimated*

Table 4. The quality of life according to the use of Tenofovir and Lamivudine (N=42)

Quality of life SF-36		Tenofovir Average±SS	Lamivudine Average±SS	t	p
Sub dimensions	Physical Function	67.67±29.15	83.89±19.82	2.143	***0.038****
	Physical Role	71.67±42.12	77.78±40.63	0.461	0.647
	Pain	75.27±18.60	76.96±18.42	0.285	0.777
	General Health Perception	62.33±21.05	66.15±26.75	0.476	0.637
	Vitality- Prostration	49.33±21.62	58.52±26.16	1.156	0.254
	Social Function	75.83±30.42	80.09±25.30	0.486	0.629
	Emotional Role	77.78±37.09	77.78±40.30	0.000	1.000
	Mental Health	69.60±19.17	69.33±18.33	0.044	0.965
Main dimensions	Physical Dimension	68.20±24.74	75.18±22.61	0.927	0.359
	Mental Dimension	68.56±23.04	71.04±23.77	0.328	0.745

**P<0.05 Statistically Significant*

Having analyzed the quality of life; the Physical Dimension point average is found as 72.69±23.34 while the Mental Dimension point average is 70.16±23.26. A significant difference is found only in physical function (PF) sub-dimension (p=0.038) among the sub-dimensions of drugs and life quality and it has been found that the ones using LAM have higher PF point average (Table 4).

Discussion

It is indicated the antiviral drugs used for the patients having chronic hepatitis B may prevent the liver-related mortality or development of hepatocellular carcinoma [9]. Although antiviral drugs generally have some side effects like prostration, high fever, headache, muscle pain, nausea, sleep withdrawal, asthenia and depression; it has been stated in literature that these side effects generally depend upon pegylated interferon (PEG-INF) [23].

The most important side effect related with TFV is stated as nephrotoxicity whereas the other ones being seen rarely are osreomalacia, diarrhea, nausea, throwing up, stomachache, extreme fatigue, headache, depression, serum amylase, and the increase of the level of triglyceride and creatinine kinase [12,23]. As well as LAM has been known as tolerated well and not causing serious side effects; it also has rare side effects as mild epigastric discomfort, diarrhea, paresthesia anorexia, nausea-throwing up, anaemia and leucopenia [24,25]**.** According to a similar study, have determined that the patients using TFV more commonly have the prostration complaints which cannot be identified prior to the treatment and can not be explained with some other causes [12]. Kose et al. (2012) have stated that when they searched for the effectiveness of TFV treatment in chronic hepatitis B (CHB) for 48 weeks, they did not encounter with major side effects throughout the treatment [26].

The psychological problems (such as depression, anger-hostility, anxiety) arising from interferons in patients with chronic hepatitis, affect success of the treatment negatively [27]. In this study, it is determined that one third of the patients are having anxiety and 40.5% of them are having

depression. Even though there is no significant difference, the rate of anxiety (16.7%) and depression (26.2%) possibility in patients using LAM has been found more than the ones using TFV. Investigating the literature; although the depression possibility due to PEG-INF use is stated as varying between 20- 40% [14,23]; no study handling psychological problems related with the use of TFV and LAM have been found.

The quality of life of the patients with chronic viral hepatitis has been affected negatively sometimes due to illness and the antiviral treatment. It has been emphasized in the applied studies that the quality of life of the patients with viral hepatitis is poor and it affects especially the physical role, energy, prostration and social function sub dimensions more [28-33]. In this study, the lowest LQ score is determined at vitality- prostration sub-dimenson. PF sub-dimension score is found as significantly lower in TFV users compared with LAM users. Similarly, in the studies of Altindag et al., it is stated that the sub-dimension scores of general health, dignity- prostration, physical role strength have lower scores [34]. And in the studies of Yi et al. (2006); the treatment-related scores of physical function, mental health, social function, physical pain and dignity are lower compared to the past [31]. Likewise; Foster et al. have also stated that there is more role limitations in patients with hepatitis C compared with the ones with hepatitis B in terms of social function, energy, prostration and physical problems [28]. In the studies of Ozkan et al. held in our country with the patients of non-cirrhotic compensated liver having hepatitis B or hepatities C; a significant importance has been found between the patients and the control group in all fields related with life quality; and it has been shown that HBV has negative effect on life quality in terms of physical function and mental health [35].

Tilmam et al. have found in their studies that the life quality scores of the patients with HCV are worse than the ones having HBV; and other liver disorders and between the ones having HBV and other liver disorders, there is no significant difference [36]. Also in another study, it has been found out that the life quality of patients is low in all subdimensions [37].

It has been determined that; between the subdimensions of drugs and life quality; the PF score averages are high only in physical function subdimension. We are on the opinion that this might be because of the fact that the patients using LAM have lower viral load.

Conclusions

According to the results; the most common side effects are prostration, muscle pain, headache and anorexia while the anxiety and depression affect the life quality negatively. We think that the education of health professionals about the new generation drugs in terms of the management of the treatment and care of the patients with viral hepatitis; and evaluating the patients not only for the effectiveness of the drugs but also for side effects, psychological problems and quality of life will increase the success and the maintability.

The limitation of the number of examples of the study to a small amount has been a significant point. For this reason, we believe that we need to have multicentered studies with a bigger example group aimed at side effects, complications, psychological problems and quality of life arising from the use of TFV and LAM.

As a result, evaluating the symptoms that may affect the life quality of patients with chronic hepatit, no significant difference has been found between two drugs except physical function subdimension. However in terms of physical function, LAM has been found as more effective

Authors' contributions

O Karabay was the senior most responsible investigator for the project who had study idea, wrote the protocol, and coordinated the project. D Aygin and H Sert wrote the first and final drafts of the manuscript. D Aygin is the corresponding author. D Aygin and H Sert conducted the statistical analyses and jointly interpreted data. O Karabay edits to the manuscript. O Sandikci carried out clinical sample collection and was responsible for clinical data. All authors read and approved the final manuscript.

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Bone mineral density correlates with t-TG levels in pediatric patients with celiac disease

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Abstract

Background and Aims: Chronic inflammation and malabsorption in celiac disease (CD) can cause bone metabolism alterations and bone mineral loss. Low bone mineral density (BMD) is a risk factor for osteoporosis and bone fracture, not only in childhood and adolescence, but also later in adulthood. The aim of the present study was to determine the extent, to which anti-tissue transglutaminase (t-TG) antibody levels correlate to bone mineral density (BMD) in children and adolescents with CD.

Methods: The study included 41 pediatric patients with CD (23 girls, 18 boys), between 10 and 18 years of age, and control group of 37 healthy subjects (19 boys, 18 girls). All patients declared to be on a strict gluten free diet (GFD) for the period of at least 6 months prior to the study. Anti-tissue transglutaminase (t-TG) level was measured in all the patients. Lumbar and left hip BMD were measured using dual-energy X-ray absorptiometry (Hologic Explorer QDR). Results are expressed as Z scores for a particular chronological age.

Results: Bone mineral density in children and adolescents with CD, who are consuming a strict GFD, was not significantly lower according to the local reference values (lumbar Z = -0.2 and left hip Z = -0.4). When compared to the control group, their BMD showed no significant difference (lumbar Z = -0.3 and left hip Z = -0.8). Even though all the patients declared to be on a strict gluten free diet, only 58 % of them had negative values of t-TG. Interestingly, we found a significant negative correlation between BMD and t-TG levels within the serologically negative CD patients, where higher levels of t-TG were associated with lower BMD (R = -0.5, p = 0.032).

Conclusion: A strict gluten-free diet is of great importance in patients with CD. It reduces the risk of long-term complications such as osteoporosis in this group of patients. Our results indicate that bone mineral density even in patients, who are consuming a strict GFD and are considered serologically negative can be affected according to the levels of t-TG which relates to the strictness of the diet. It is therefore strongly recommended that all CD patients comply with gluten free diet as strictly as possible.

Key words: Bone mineral density, celiac disease, gluten free diet, children and adolescents, tissue transglutaminase.

Introduction

Celiac disease (CD) is a chronic systemic immune mediated disease caused by intolerance to gluten in genetically predisposed individuals after exposure to gliadin and prolamin peptides present in wheat, rye and barley (1,2). It is characterized by a complex interplay between genetic and environmental factors and is commonly presented by immune-mediated enteropathy, associated with maldigestion and malabsorption of many vitamins and other nutrients (3,4). Recent developments in proteomics have provided an important contribution to the understanding of biochemical and immunological aspects of celiac disease and the mechanisms involved in the toxicity of prolamins. Furthermore, it has been demonstrated that some gliadin peptides, resistant to complete proteolytic digestion may directly affect intestinal cell structure and functions by modulating gene expression and oxidative stress (5). Celiac disease affects children and adults at rates approaching 1 % of European and American population (6,7). However, recent studies showed an increase in prevalence of celiac disease in European countries (8). In the rest of the world celiac disease is less commonly diagnosed, usually due to the healthcare system but the prevalence is estimated to be equal to that of Europe and USA. The only exception is Sub-Saharan Africa, where prevalence

of celiac disease reaches almost 5 % of the population (9).

The prevalence of CD among first-degree relatives is much higher than the prevalence of the disease in the general population. Most of these patients have an atypical presentation of the disease and would be therefore overlooked without an active search (10). CD is strongly associated with HLA-DQ2 and/or HLA-DQ8, as both genotypes predispose for the disease development (11). For that reason, serological testing is recommended for all first-degree relatives of CD patients. In addition, they should undergo HLA typing to detect those whose HLA phenotype is consistent with CD. This approach can also help in excluding individuals who do not need further diagnostic procedures (10). Currently, serological screening tests are utilized primarily to identify those individuals in need of a diagnostic endoscopic biopsy, although there is a possibility to diagnose the disease solely based on serology according to latest guidelines of European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) (12). Ultrasonography may also provide valuable information on small-bowel wall structure and can help in decision making on the necessity of small bowel biopsy. However, there is a much stronger correlation between serological markers and CD then between ultrasonography and CD (13). The determination of serum levels of IgA anti-tissue transglutaminase antibodies (t-TG) is the first choice in screening for celiac disease (12).

Evidence indicates that there is a well-established relationship between low bone mineral density (BMD) and CD. However, the exact pathogenic mechanisms are still inconclusive (14,15,16). Reduced BMD is frequently found in individuals with celiac CD, who are not consuming a strict gluten free diet (GFD), possibly due to calcium and vitamin D malabsorption, release of pro-inflammatory cytokines, and misbalanced bone remodeling (17). In patients with symptomatic CD, low BMD appears to be directly related to the intestinal malabsorption, which is well documented consequence of CD. The impairment of absorption of calcium in celiac disease is a consequence of intestinal villous atrophy in proximal intestine, where majority of calcium is being absorbed. A strict GFD may reverse intestinal villous atrophy and improve absorption (18).

Furthermore, during the last two decades, the effect of CD on bone health has been extensively studied (2,19). Celiac disease is often overlooked (or simply forgotten) as a cause of osteoporosis. Yet, the consequences of osteoporotic fractures can be devastating (20,21). Bone remodelation is under constant hormonal influence and for that reason pediatricians pay a lot of attention to hormones and growth factors in children and adolescents. Secondary hyperparathyroidism may occur as a consequence of hypocalcaemia. In the study from 2008, Zanchi et al. found that children with CD had higer levels of PTH when compared to the control group but were normalized after six months of GFD (22). Two recent studies of Garrote et al. and Mora analyzed a complex system of cytokines involved in the pathogenesis of CD and described certain amount of interferon gamma (IFN-γ) in intestinal mucosa together with increased production of interleukines IL-15, IL-18, and IL-21 related to ingestion of gluten. An increased production of inflammatory cytokines and at the same time presence of specific autoantibodies can provoke a disruption of bone metabolism equilibrium in children and adolescents with CD. Even though the research is scarce, the evidence show that inflammatory factors are involved in pathogenesis of bone mineralization (23, 24).

Tissue transglutaminase is a crucial factor in CD because it promotes the gluten-specific T-cell response and is also the target of the autoimmune response. Tissue transglutaminase induces changes in gluten, which in turn, causes the generation of a series of gluten peptides that bind to HLA-DQ2 or DQ8 molecules with high affinity. The resulting HLA-DQ2 HLA-DQ8-gluten peptide interaction triggers the proinflammatory T cell response. Tissue transglutaminase is also involved in other non-T-cell-mediated biological activities of gliadin peptides (25). In addition, the anti-endomisial antibodies (EMA) are frequently used in the diagnosis of CD, and positive EMA is a very strong predictor of a disease (26). Once the diagnosis has been established, serological testing is being routinely used in the monitoring of the response to a gluten-free diet (12, 27, 28). There is evidence that decreased concentrations of antibodies are significantly associated with the degree of compliance with the GFD. Among others, t-TG

has the best and most consistent performances. The serial measurement of antibody levels seems to be more reliable in monitoring compliance than the positive/negative expression of results (29, 30). Periodical serological and clinical follow-ups are a viable and efficacious strategy to promote adherence to gluten-free diet (31, 32).

At the moment the only efficient treatment of CD consists of a strict, lifelong GFD, which promotes an improvement of the disease activity which yields to the recovery of bone mineralization in children (17). Recent studies confirmed the importance of complying with strict GFD in children and adolescents with CD until the end of skeletal mineralization even when patients are asymptomatic (1, 33). A strict gluten-free diet improves bone mineralization and marked improvements in BMD can be seen already during the first year of the introduction of GFD (34, 35). However, it is very difficult to achieve a strict GFD since food often contains traces of gluten. Moreover, the general use of wheat and wheat starch in production lines in the industry poses a threat of contaminating other, previously gluten free products (36). Because of the difficulty of acquiring gluten free nutrition, a gluten free product is a product that is tolerated by great majority of CD patients. With the development of new methods for gluten detection, the limits for gluten presence in food products are changing. By the Codex Alimentarius standard from 2008, gluten free nutrients are those that contain less than 20 mg/kg of gluten. In this way, patients on strict GFD ingest less than 10 mg of gluten daily (37). Children with CD have to be followed-up regularly for the growth control, development as well as GFD compliance. The latter is verified by controlling EMA and t-TG levels in CD patients. Low titers of antibodies indicate appropriate diet compliance and hence proper treatment. It is recommended to test EMA and t-TG antibodies at least once a year (38).

While other studies mostly focused on bone mineral density in CD patients with emphasis on GFD, vitamin D and calcium, we tried to evaluate the correlation between CD and t-TG as a specific serological marker for the GFD adherence and marker of the disease activity. The aim of the present study was to evaluate the differences in BMD between CD and control group. Furthermore, BMD was compared to t-TG levels in CD patients following GFD and the relation between t-TG and BMD was assessed only within the group of serologically negative CD patients (t-TG < 100 U/ml). To our best knowledge, no other study directly analyzed the correlation between t-TG levels and BMD in serologically negative CD patients.

Patients and methods

Patients

In the present prospective study, which was conducted between March 2011 and April 2012, a total of 78 children and adolescents between 10 and 18 years of age were selected and followed-up. Pediatric patients with celiac disease were diagnosed according to ESPGHAN diagnostic criteria from 1990, which include positive values of serological markers and histological demonstration of villous atrophy in duodenal mucosa (12). The subjects were thereafter divided into two groups: children and adolescents with CD (n = 41; 23 girls, 18 boys), and a healthy control group (n = 37; 19 boys, 18 girls). All CD patients were recruited during their regular follow-up visits. All were Caucasians residing in North-Eastern Slovenia. All patients declared to be consuming a gluten-free diet (GFD) for the period of at least six months prior to the study. None of the patients was previously treated with mineral or vitamin supplements. After a written consent was obtained from parents or children if they were old enough, children/adolescents were included in the study.

The control group consisted of healthy children and adolescents of the same age and same geographical area (n = 37; 19 boys, 18 girls). Nine of them were voluntarily recruited from the general population but the most of them were recruited from the Pediatric clinic during their check-up visits following their previous hospitalization due to a minor acute disease (most commonly abdominal colic, diarrhea, and urinary tract infection). None of them had any previous or present suspicion for a chronic gastrointestinal disorder.

However, two participants with CD did not reach inclusion criteria and were for that reason excluded from the study. The reasons for exclusion were hypertension (1 adolescent) and chronic kidney disease (1 adolescent). In addition, 5 children

and adolescents from the control group refused to participate, while 8 of them agreed to participate but after clinical and laboratory work-up they did not complete the appointed DXA measurement.

Parent(s) and child/adolescent received all the necessary information regarding the study. After obtaining the written consent, participants were included in the study. Following the interview with all the participants, clinical examination was performed.

Body height and weight were measured and samples of venous blood taken for investigation of serological markers. Afterwards, participants were appointed to BMD measurement using DXA. All diagnostics were performed at the time of inclusion in the research.

The study was approved by the National medical ethics committee.

Serological markers

Antibodies against tissue transglutaminase (t-TG)

The levels of IgA anti t-TG antibodies were determined at the Department of laboratory diagnostics at University Medical Centre Maribor using standardized methods (Luminex, AtheNA Multi-Lyte® Celiac Plus Assay, The Zeus Scientific). Cut off values were 100 U/ml where higher values were considered positive. According to manufacturer, the sensitivity and specificity of IgA antibodies against t-TG were 93.1 % and 77.3 % respectively (39).

Antiendomysium antibodies (EMA)

The levels of IgA antiendomysial antibodies were also determined at the Department of laboratory diagnostics at University Medical Centre Maribor using standardized methods (Antiendomysium 96, Eurospital). Results were interpreted as positive or negative by medical personnel. As stated in studies cited by manufacturer, specificity of IgA antibodies against antiendomysium was 98% and specificity 99% (40).

Dual-energy X-ray absorptiometry

Bone mineral density (BMD) was measured at the lumbar spine (L1-L4) and total hip using dual-energy X-ray absorptiometry with the same instrument (Hologic Explorer QDR) and by the same medical personnel. The device and the system were calibrated daily. BMD was expressed as Z-score that was determined from local reference data. In bone mineral densitometry, normal BMD is defined as T-score $\geq$ -1, osteopenia is defined as Z-score < -1 and > -2.5, and osteoporosis as Z-score $\leq$ -2.5 (25).

Bone age

Bone or skeletal age was determined in all patients in order to assess their chronological bone development. X-ray imaging and interpretation were conducted on left hand and wrist at the Department of radiology at University Medical Centre Maribor. Bone age was then compared to chronological age and result expressed as difference between the two.

Statistical methods

SPSS v. 16.0.1 was used to determine the relationship between bone mineral density and serological markers (t-TG). Graphs were plotted to illustrate the relation of bone mineral density and t-TG and ANOVA was used for calculation of coefficients and significance. For statistical analysis, the patients were divided into two different groups based on the levels of t-TG: a) subjects compliant with GFD and b) those that were not compliant. Afterwards, the group of serologically negative CD patients was further analyzed to determine the quantitative levels of t-TG. Bone mineral density was then compared to the levels of t-TG and correlation was calculated using ANOVA test.

Results

Even though all patients included in the present study declared to be on a gluten-free diet for the period of at least six months prior to the study; 41 % (n=17; 9 girls, 8 boys) of children and adolescents from the study were serologically positive, which means they had a level of t-TG antibodies higher than 100 U/ml. The mean t-TG level in the group of CD patients was 139 ± 162 U/ml.

Bone mineral density was measured in all subjects, both CD patients and healthy control group, and expressed as Z score determined from local reference data. The results showed that mean lumbar bone mineral density for CD group was -0.1 and left hip BMD -0.4 (n = 34). When the-

se mean values were compared with the subjects from the control group with mean lumbar Z = -0.3 and left hip Z = -0.8 (n = 29), no significant difference from the CD group could be observed. The same result with mean bone mineral density (Z score) in CD group was found when compared to the control group, where difference did not reach statistical significance with lumbar spine Z p=0.588 and left hip Z p = 0.108 (Table 1).

Table 1. Bone mineral density in celiac disease and control group

Diagnosis	Lumbar Spine BMD (Z score)	Left Hip BMD (Z score)
Celiac disease	-0.1	-0.4
Control group	-0.3	-0.8
p	0.588	0.108

Chronological bone development was assessed by comparing the difference in bone and chronological age between different groups of patients. Mean bone and chronological age difference was 0.223 ± 0.68 (n = 70), while mean values were 0.197 ± 0.77 (n = 34) for CD and 0.247 ± 0.58 (n = 36) for healthy control group respectively. There was no significant difference between CD patients and their healthy peers (p = 0.760). However, correlation was found between bone age and lumbar spine BMD (p = 0.042), while the correlation between bone age and left hip BMD did not reach statistical significance (p = 0.348).

CD patients were divided into two groups based on t-TG levels with cut off value of 100 U/ml. Patients with low t-TG levels thus considered serologically negative had mean lumbar Z = -0.2 and mean left hip Z = -0.4 (n = 19) while serologically positive group had mean lumbar Z = 0.0 and mean left hip Z = -0.3 (n = 15). No significant difference could be found in BMD between serologically positive and negative group with significance p = 0.520 for lumbar Z and p = 0.660 for left hip Z respectively. This was repeated after dividing the patients according to their EMA measurement. For EMA negative group mean lumbar Z = -0.1 and mean left hip Z = -0.4 (n = 26) while for EMA positive group mean lumbar spine Z = -0.4 and mean left hip Z = -0.3 (n = 7). No significant difference was found in BMD between EMA positive and negative group with p = 0.391 for lumbar spine Z and p = 0.952 for left hip Z.

Later, we focused on t-TG negative group of celiac disease patients (t-TG < 100 U/ml), who declared to be following a strict gluten free diet. Low levels of t-TG antibodies were considered to be a good indicator of diet compliance and therefore this group of patients had been actually serologically proven to be GFD compliant. Group of t-TG negative CD patients consisted of 22 patients (13 girls and 9 boys, with mean age 15 ± 2.6 years). Mean t-TG level of serologically negative group was 38 U/ml ± 27 U/ml. Focused analysis, however revealed a marked negative correlation between BMD and serum t-TG levels where patients with lower t-TG levels achieved higher BMD scores than those closer to the upper limit of t-TG. Detailed analysis showed that the correlation between lumbar spine BMD and t-TG levels was statistically significant (R = -0.5, p = 0.032) (Figure 1). Attempts were made to find a correlation between BMD and left hip, however the correlation did not reach statistical significance (p = 0.119).

Discussion

Chronic inflammation and malabsorption in celiac disease (CD) is known to cause bone metabolism alterations and bone mineral loss, which can lead to osteoporosis and an increased risk of bone fractures later in life (1, 34). The only efficient treatment of CD thus far is a strict gluten-free diet (GFD). Therefore, a strict compliance to the diet is of great importance for the patients' wellbeing and should be evaluated by their physicians regularly (1, 15, 33). Serological markers (both t-TG and EMA) were found to be good markers for the objective determination of the patients` compliance with GFD (12, 27). Studies outline both t-TG and EMA to be useful tool for both diagnosis of CD and follow-up (12, 27, 41).

Contrary to other studies, when investigating the differences in BMD between CD and healthy control group, we found BMD in celiac patients tending to be higher than in the control group (1, 16, 33). Even though the difference did not reach statistical significance, the result implies that celiac patients are not at an increased risk for osteoporosis and its complications. This higher than expected BMD could be explained as a result of a better knowledge about nutrition and healthy and well balanced diet, since a lot of effort is put into

education of CD patients about their disease with a lot of emphasis on the importance of nutrition, physical activity and a healthy life style.

In addition, bone age was analyzed in all groups of children. Both groups of CD patients and control group had mean bone age slightly ahead of chronological age. This implies that both groups were appropriately developed according to the skeletal bone growth. While comparing both groups, no difference was found. This is consistent with previous results of BMD in different groups and confirms that their skeletal system is both chronologically well developed as well as sufficiently mineralized. While analyzing bone age, we found statistically significant correlation with lumbar spine BMD. There are no other studies that we know of, that investigated usefulness of bone age measurement in pediatric CD patients, yet our findings suggest bone age might also be a fast and inexpensive tool for prediction of low BMD and thus related risks.

Furthermore, we investigated the effect of elevated t-TG levels on BMD in children and adolescents with CD. However, no statistically significant difference was found when comparing BMD between the groups with t-TG positive and t-TG negative levels, which could be a result of relatively small sample of patients. The same analysis was repeated after separating CD patients in groups of EMA positive and EMA negative patients. However, no significant difference was found. Further studies are needed with larger groups of patients.

We hypothesized there should be a correlation between t-TG and BMD since serological markers are well accepted tool for monitoring the disease and there are plenty of evidence regarding the effect of CD on BMD (1, 14, 15, 34, 41, 42). Low BMD may be a result of intestinal malabsorption in symptomatic CD. Intestinal villous atrophy in proximal intestine and therefore malabsorption of calcium is highly related to strict GFD which may reverse the process (18). Since there is evidence that decreased concentrations of t-TG antibodies are significantly associated with the degree of compliance with the GFD, correlation between t-TG and BMD is also expected (29, 30). To our best knowledge, not many studies had focused directly on that particular relation, yet Kocsis et al. had mentioned a correlation between the two in his observation of a single coeliac centre (43).

It is reasonable to expect the difference in BMD between the group of patients on a strict GFD and those who are not on GFD. Patients who were complying with the strict GFD and were checked regularly by their pediatrician (with both clinical examination and laboratory studies), were therefore considered as being appropriately treated and expected to be at equally low risk for reduced BMD. However, when analyzing the results we found a difference in BMD within the group of children that were considered to be on a strict GFD by self-reported dietary history, and who also had levels of t-TG within the normal range. We discovered that there was a significant difference among the patients who were considered as being well treated and appropriately complying with GFD. Statistically significant correlation was found between BMD and the levels of t-TG. Those with low t-TG levels had significantly better BMD than those with higher t-TG levels while still being low enough to be considered serologically negative ($p = 0.032$). Our results are in agreement with Kocsis' study, in which a correlation has been established between BMD and t-TG (43). Agardh's study showed similar results yet with different antibodies, while Margoni's study stated that no biochemical marker is capable of predicting an abnormal BMD (44, 45). No other study that we know of focused on a group of t-TG negative patients and presented t-TG as a tool for detecting a decreased BMD in CD patients who were considered well treated.

This raises several questions regarding the appropriate definition of reference values, as well as the use of serological markers as an objective tool for evaluating treatment success. It shows that not merely the diet itself, but also the strictness of the diet is important and the consequences can be reflected on the bone mineral density. The risk of all future complications of CD, not merely risk of bone fractures, may very likely be related to the strictness of the diet in a much greater way than it was previously suspected. The results of our study should encourage all physicians dealing with celiac disease to advise their patients to follow a strict gluten-free diet as much as possible. Physicians should not have the reference values of serological markers as the goal of the treatment but rather tend to achieve the diet as strict as possible

and have the serological markers the lowest possible. Only in that way can we be certain that patients are getting the best possible treatment.

Conclusion

Patients with celiac disease on a strict gluten free diet are not considered to be at an increased risk for osteoporosis or bone fractures due to a good bone mineral density. This study shows the importance of the strictness of the gluten-free diet in children and adolescents with celiac disease. Even within the group of patients with CD, who declared to be on a strict GFD, we found a spectrum of strictness of the diet which influenced their BMD accordingly. This finding raises the importance of a strict gluten free diet.

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Cardiovascular manifestations in pre and postmenopausal breast cancer patients

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Abstract

Background: Breast cancer patients are at increased risk of death not only from cancer but also from other major competing causes of mortality, particularly cardiovascular disease, which is associated with altered lipid profile. The present study was conducted to identify the breast cancer patients on cardiovascular risk.

Methods: Serum lipid profiles in premenopausal and postmenopausal breast cancer patients, before and after chemotherapy, and their comparable controls were investigated. Fasting blood samples of controls and patients, at diagnosis of disease and after chemotherapy, were processed for serum and analyzed.

Results: Serum total lipids (TL), triglycerides (TG), cholesterol, and low-density lipoproteins (LDL) elevated significantly ($P<0.05$), whereas, high-density lipoprotein (HDL) indicated a significant decline ($P<0.05$), in women with breast cancer than comparable controls. The alterations were more pronounced in post compared to premenopausal breast cancer patients and controls.

Conclusions: Evaluation of serum lipid profile could be considered as a vital clinical index for breast cancer diagnosis and prognosis. It is recommended that lipid profile should be estimated in postmenopausal women presenting with breast cancer to avert the risk of cardiovascular disease in these patients.

Key words: Breast cancer, cardiovascular disease, chemotherapy, postmenopausal, serum lipid profiles.

Introduction

The prevalence of metabolic syndrome like obesity, glucose intolerance, low serum high-density lipoprotein cholesterol, high serum triglycerides and hypertension is high and increasing in parallel with an increasing breast cancer incidence worldwide (1). Elevated lipid levels precede the development of the breast cancer and thus may have an etiological or predictive significance (2). Until now, little is known and conflicting results have been reported on the relationship between serum lipids and risk of breast cancer in women (3). Pretreatment lipid profile of breast cancer patients' revealed elevation of serum triglycerides and total cholesterol (4) but controversy is still present.

Several clinical studies suggest the prognostic significance of serum lipid levels in breast cancer. Levels of circulating lipids and lipoproteins have also been associated with breast cancer risk, though published results have been inconsistent (5). Therefore, it is still necessary to further clarify any association of lipid profile with breast cancer risk, especially in Asian population. Therapy for cancer has progressed dramatically in recent years, and tremendous progress has been made in reducing the morbidity and mortality from many forms of cancer. The therapeutic options for patients with cancer now include increasingly complex combinations of medications, radiation therapy and surgical intervention. Many of the above mentioned therapeutic measures have important potential adverse cardiac effects and are likely to have significant effects on patient outcomes. Combination therapy became the standard of care for women with a 90% objective response rate with the Five Drug Combination (FDC) of chemotherapeutic drugs. Multiple chemotherapeutic agents have been used successfully in the treatment of breast cancer (6).

The present study was therefore, planned to evaluate the relationship of breast cancer with the lipid profile of the patients, to investigate the prognostic significance of the lipids in the early diagnosis of breast cancer in the local population and to investigate the risk of breast cancer in pre and postmenopausal women with the increased body weight. In view of the increased emphasis on the use of FAC (5-Flouro uracil/ 5FU, adriamycin,

cyclophosphamide) regimen as chemotherapeutic agent for the treatment of breast cancer, it is imperative to investigate the effects of these drugs on the lipid profile of the breast cancer patients during chemotherapy and association of such altered lipid profile with possible cardiovascular manifestations in these patients.

Methods

Patients and control subjects

The study was approved by Ethical Review Committee (ERC) of University of the Punjab, Lahore. Samples of breast cancer patients were collected from Institute of Nuclear Medicine and Oncology (INMOL), Lahore, from October 2010 to March 2012. Initially 102 newly diagnosed breast cancer patients were recruited for the study, out of which 84 patients fulfilled the criteria and were selected as participants of the study. Consecutively, during the study period, 22 more newly diagnosed breast cancer patients were included in the study, thus making a total of 106 samples of patients. All breast cancer cases were of the histological grades 2 and 3 (World Health Organization [WHO] Classification of Breast Tumors, 1981). They were grouped according to their menopausal status i.e. premenopausal (n=44) and postmenopausal (n=62). The participation rate was 74% in controls (n=52) and 96% in breast cancer patients.

Control subjects were randomly selected as a reference population for comparative purpose. They were also grouped according to their menopausal status i.e. premenopausal (n=24) and postmenopausal (n=28). They were all healthy, non-pregnant, non-smokers and without any previous history of chronic disease. All of the participants belonged to the same socio-economic status and age groups (26-63 years). The objectives of the study were explained to each woman and a written consent was obtained.

A detailed clinical history of lipid abnormalities of each of the patients was recorded on a proforma designed for the study. History of pregnancy, usage of oral contraceptives, hormonal medications, surgery and use of other chemotherapeutic drugs was recorded. Women presenting with pregnancy, hypertension, diabetes mellitus, cardiovascular disease, or those on any form of drug therapy were excluded from the study. The blood samples of the patients were obtained after an overnight fast (12-16 hours) before chemotherapy and twenty days following five courses of chemotherapy.

Study protocol

The women were interviewed and clinically examined for the signs and symptoms of breast cancer by qualified medical personnel. The diagnosis was based on clinical history, general physical examination, mammography and biopsy measurements.

Laboratory analysis

Lipid analyses were carried out on serum samples from 106 breast cancer patients and 52 control subjects. Fasting blood samples were processed to obtain serum after centrifugation at 3000rpm for 15 minutes and were preserved at -70°C before further examination. Samples were analyzed for serum lipid profile using commercially available kits for total lipids (Spinreact, Spain. Ref no. 1001270), triglycerides, cholesterol, low density lipoproteins and high density lipoproteins (Chema Dianostica, Italy.) by using clinical chemistry analyzer (Model 5010, Robert Riele GmbH & Co KG. D-13467 Berlin, Germany).

Statistical analysis

Statistical analysis was made by using GraphPad Prism version 5.00 (San Diego California, USA). Data was presented as Mean ± SEM, analyzed statistically using one-way ANOVA and Tukey's Post Hoc Multiple Comparison test and employed in comparing the variations amongst the cohort of the study. Differences were considered to be significant at $P<0.05$.

Results

Control premenopausal vs premenopausal pre-treated subjects

A significant increase of 50% ($P<0.001$) for serum levels of total lipids, 61% ($P<0.01$) for triglycerides, 17% ($P<0.001$) for cholesterol and 31% ($P<0.01$) for LDL was observed in pre-menopausal pretreated subjects when compared with control premenopausal subjects, while there was a significant decrease of 22% ($P<0.01$) in serum

HDL levels in premenopausal pretreated subjects in comparison to control pre-menopausal subjects (Table 1 & 2).

Control premenopausal vs premenopausal post-treated subjects

There was a significant increase of 49% (P<0.001), 86% (P<0.001), 24% (P<0.01) and 41% (P<0.001) in serum levels of total lipids, triglycerides, cholesterol and LDL, respectively, in premenopausal pre-treated subjects in comparison to premenopausal control subjects. Moreover, a significant decrease of 33% (P<0.01) in serum HDL levels was observed in premenopausal pre-treated subjects when compared with premenopausal control subjects (Table 1 & 2).

Control postmenopausal vs postmenopausal pre-treated subjects

When postmenopausal pre-treated subjects were compared with postmenopausal control subjects, a significant increase of 51% (P<0.001), 50% (P<0.001), 28% (P<0.001) and 34% (P<0.01) in serum levels of total lipids, triglycerides, cholesterol and LDL, respectively, was found in postmenopausal pre-treated subjects. A significant decrease of 35% (P<0.001) in serum levels of HDL was observed in postmenopausal pre-treated subjects in comparison to postmenopausal control subjects (Table 1 & 2).

Table 1. Average levels of serum lipid profile (mg/dL) in various groups of study. Values are mean±SEM

	Con PreM	Con PostM	PreM PreT	PreM PostT	PostM PreT	PostM PostT
Total Lipids	467.5 ± 18.1	499.1 ± 15.2	702.9 ± 17.9	698.9 ± 31.3	752.9 ± 16.4	798.9 ± 9.8
Triglycerides	79.2 ± 6.7	90.6 ± 5.7	127.8 ± 15.6	147.1 ± 2.4	135.9 ± 10.9	183.3 ± 12.1
Cholesterol	152.4 ± 6.6	167.5 ± 5.1	178.1 ± 5.9	188.3 ± 4.2	213.6 ± 14.6	226.8 ± 5.9
LDL	58.2 ± 2.4	71.9 ± 3.2	76.5 ± 3.6	82.2 ± 2.4	96.0 ± 3.8	99.0 ± 6.8
HDL	46.2 ± 2.6	36.4 ± 1.9	36.0 ± 1.1	30.8 ± 2.2	23.7 ± 3.5	19.5 ± 2.2

Con	*Control*
PreM	*Pre-menopausal*
PostM	*Post-menopausal*
PreT	*Pre-treated*
PostT	*Post-treated*
*, **, ***	*P<0.05, 0.01, 0.001, respectively*
↑, ↓	*Increase, Decrease*

Table 2. Percentage variations of serum lipid profile in comparable groups

	Con PreM vs PreM PreT	Con PreM vs PreM PostT	Con PostM vs PostM PreT	Con PostM vs PostM PostT	PreM PreT vs PostM PreT	PreM PostT vs PostM PostT	PreM PreT vs PreM PostT	PostM PreT vs PostM PostT
Total Lipids	50 ↑ ***	49 ↑ ***	51 ↑ ***	60 ↑***	7 ↑ **	14 ↑ *	1 ↓	6 ↑*
Triglycerides	61 ↑ **	86 ↑ ***	50 ↑ ***	102 ↑ ***	6 ↑ *	25 ↑ *	15 ↑*	35 ↑*
Cholesterol	17 ↑ ***	24 ↑ **	28 ↑ ***	35 ↑ ***	20 ↑ **	20 ↑ *	6 ↑*	6 ↑*
LDL	31 ↑ **	41 ↑ ***	34 ↑ **	38 ↑ ***	25 ↑ **	20 ↑ **	7 ↑*	3 ↑
HDL	22 ↓ **	33 ↓ **	35 ↓ ***	46 ↓ ***	34 ↓ ***	37 ↓ ***	14 ↓*	18 ↓*

Con	*Control*
PreM	*Pre-menopausal*
PostM	*Post-menopausal*
PreT	*Pre-treated*
PostT	*Post-treated*
*, **, ***	*P<0.05, 0.01, 0.001, respectively*
↑, ↓	*Increase, Decrease*

Control postmenopausal vs postmenopausal post-treated subjects

A significant increase of 60% (P<0.001) for serum levels of total lipids, 102% (P<0.001) for triglycerides, 35% (P<0.001) for cholesterol and 38% (P<0.001) for LDL was observed in postmenopausal post-treated subjects when compared with postmenopausal control subjects. While there was a significant decrease of 46% (P<0.001) in serum HDL levels in post-menopausal post treated subjects in comparison to postmenopausal control subjects (Table 1 & 2).

Premenopausal pre-treated vs postmenopausal pre-treated subjects

There was a significant increase of 7% (P<0.01), 6% (P<0.05), 20% (P<0.01) and 25% (P<0.01) in serum levels of total lipids, triglycerides, cholesterol and LDL, respectively, in postmenopausal pre-treated subjects in comparison to premenopausal pre-treated subjects. Moreover, a significant decrease of 34% (P<0.001) in serum HDL levels was observed in postmenopausal pre-treated subjects when compared with premenopausal pre-treated subjects (Table 1 & 2).

Premenopausal post-treated vs postmenopausal post-treated subjects

A significant increase of 14% (P<0.05) for serum levels of total lipids, 25% (P<0.05) for triglycerides, 20% (P<0.05) for cholesterol and 20% (P<0.01) for LDL was observed in postmenopausal post-treated subjects when compared with premenopausal post-treated subjects. There was a significant decrease of 37% (P<0.001) in serum HDL levels in postmenopausal post-treated subjects when compared with premenopausal post-treated subjects (Table 1 & 2).

Premenopausal pre-treated vs premenopausal post-treated subjects

When premenopausal post-treated subjects were compared with premenopausal pre-treated subjects, a significant increase of 15% (P<0.05), 6% (P<0.05) and 7% (P<0.05) in serum levels of triglycerides, cholesterol and LDL, respectively, was found in premenopausal post-treated subjects. While serum total lipids did not vary in this comparison. There was an observed significant decrease of 14% (P<0.05) in serum HDL levels in premenopausal post-treated subjects in comparison to premenopausal pre-treated subjects (Table 1 & 2).

Postmenopausal pre-treated vs postmenopausal post-treated subjects

There was a significant increase of 6% (P<0.05), 35% (P<0.05), 6% (P<0.05) and 3% (P<0.05) in serum levels of total lipids, triglycerides, cholesterol and LDL, respectively, in postmenopausal post-treated subjects in comparison to postmenopausal pre-treated subjects. A significant decrease of 18% (P<0.05) in serum HDL levels was found in postmenopausal post-treated subjects when compared with postmenopausal pre-treated subjects (Table 1 & 2).

Discussion

Changes in lipid profile have long been associated with malignancies as lipids play a key role in maintenance of cell integrity (7). Human mammary tissue metabolizes lipids from plasma, a process affected by female gonadal hormones. Both benign and malignant proliferation of breast tissue in women has been associated with changes in plasma lipids and lipoprotein levels (8). The relationship between lipids and breast cancer is obscure. In addition, increased incidences of cardiovascular complications have been observed in postmenopausal women in comparison to premenopausal women. Therefore, the present study is aimed at comparing the serum level of total lipids (TL), triglycerides (TG), cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) between premenopausal and postmenopausal breast cancer patients, its association with developing cardiovascular disease and effects of chemotherapy (FAC regimen) in treatment of breast cancer.

The lipid profile generally increases in both breast cancer patients and controls with the patients having a much higher value than the corresponding control. LDL increases significantly only in the postmenopausal phase in comparison to the controls while cholesterol and TG increases in both premenopausal and postmenopausal phases (9). Our study has demonstrated a marked elevation of serum lipid concentrations in both the premenopausal and postmenopausal breast cancer patients com-

pared with healthy subjects. The alterations in lipid profile have been found to be more pronounced in postmenopausal compared to premenopausal breast cancer patients. Chemotherapy could not restore the levels but further intensified the response. There was a consistent increase in serum levels of TL, TG, cholesterol and LDL, whereas, a marked decrease in HDL in breast cancer patients was noticed when pre-treated samples were compared with post-treated samples. The responses were more pronounced in postmenopausal than in premenopausal breast cancer patients as well as controls.

Elevated lipid levels, preceding the development of disease, may reflect some underlying metabolic disturbance associated directly with the disease or with some hormonal factors related to the disease (10). The effect of the hormonal changes associated with menopause on the serum lipid levels play important role in most cardiac related disorders associated with menopause (11). Low serum HDL levels seem to be even a stronger predictor of cardiovascular mortality than elevated LDL cholesterol levels in postmenopausal women (12). Hypercholesterolemia is known to be a key factor in the pathophysiology of artherosclerosis (13). High levels of LDL and low levels of HDL are strongly associated with the risk of coronary artery disease (CAD). Smaller LDL particles are considered more atherogenic than larger more buoyant species because of their increased susceptibility to oxidation and their increased residence time in plasma. Plasma triglycerides concentration also has a determinative influence on the concentration of small dense LDL particles (14). As the incidences of coronary heart disease have been observed to be increased in postmenopausal women, the patients with breast cancer are at risk for death not only from cancer or its complications but also from the other major competing causes of mortality in postmenopausal women, particularly, cardiovascular disease (15). However, it is still controversial that whether dyslipidemia leads to significant increase in the development of CAD (16).

The elevated levels of triglycerides in both pre and postmenopausal breast cancer patients in comparison to controls indicates that many tumor cells are rich in intracellular neutral lipids which might be released in circulation upon cell lyses (9). However, it is unlikely that tumor cell population could contribute continuously an amount of triglycerides necessary to markedly increase serum triglyceride levels. Furthermore, as have been well documented in both animal and man, serum lipid changes may occur when tumor comprises less than 1% of the host body weight. Therefore, bulk excretion of lipids by tumor as primary cause of hypertriglyceridemia is unlikely (17). The exact mechanism of hypertriglyceridemia and decreased HDL concentration in breast cancer patients is not known. However, it has been suggested that lipoprotein lipase (LPL) may regulate the clearance of TG from blood to tissue and its activity in white adipose tissue decreases in cancer hosts, contributing to the hypertriglyceridemia (18).

The serum levels of cholesterol are significantly higher in postmenopausal than in premenopausal cases that show a capacity of serum cholesterol to become elevated in breast cancer patients (9). Higher concentrations of cholesterol and TG may either play a role in carcinogenesis or are responsible for higher incidence of breast cancer. Lower status of vitamin C in breast cancer may be one of the possible causes for the higher plasma cholesterol and TG concentrations. At the same time lower levels of vitamin C are associated with decreased plasma HDL in breast cancer patients (19). As the precursor particles of HDL are thought to derive from lipolysis of TG by LPL, increased plasma TG may be one of the factors for lower HDL concentration since the LPL activity decreases in cancer due to the ability of vitamin C to modulate the activity of LPL (20).

The exact mechanisms by which lipids and lipoproteins contribute to carcinogenesis are not clearly understood. However, reports suggest that lipid peroxidation (LPO) product, malondialdehyde (MDA), may cross-link proteins and DNA on the same and opposite strands (21). MDA forms adducts with DNA adenine and cytosine, which contributes to the carcinogenecity and mutagenecity in mammalian cells (22)34. L.J. Marnett and M.A. Tuttle, Comparison of the mutagenecity of malondialdehyde and the side-products formed during its chemical synthesis. *Cancer Res* (1980), pp. 276–282. View Record in Scopus | Cited By in Scopus (40). The LDL is more susceptible to oxidation in various pathologic conditions resulting in higher LPO during oxidative stress (23). The HDL, on the other hand, has a great potential to counter the oxi-

dative damage of LDL in cell membrane and thereby preventing LPO. The present study indicated an increase in serum LDL while serum levels of HDL were significantly lower, in both pre and postmenopausal breast cancer patients than in controls, both showing no marked recovery after chemotherapy (24). This decreased HDL in breast cancer is due to increased catabolism of HDL via increased hepatic lipase activity, reduced lecithin, cholesterol acyltransferase activity or reduced Apo-lipoprotein A-I (25). The elevated LDL and the reduction of cardio protective HDL, after menopause, is an indication that menopause is an independent risk factor for developing cardiovascular disease in breast cancer patients than in controls (12).

It has been postulated that changes in concentration of serum lipids in breast cancer could result from increased production of tumor necrosis factor α (TNFα) by activated macrophages in response to tumor. This would impair catabolism of very low density lipoprotein (VLDL), leading to an increased triglycerides and a decrease in HDL cholesterol through the reduced availability of apolipoproteins for HDL formation (26).

The past half century has seen the emergence and evolution of new therapeutic approaches for breast cancer, including chemotherapy, radiotherapy and conservative surgery (27). The FAC regimen (5-florouracil/ FU 500mgm–2, doxorubicin 50mgm–2 and cyclophosphamide 500mgm–2, all 3 weekly) is mostly used as chemotherapeutic agent in our population. However, several of other chemotherapeutic medications are now indicated to be superior in their activity and efficacy for the treatment of metastatic breast cancer e.g., a combination of taxanes and paclitaxel, TAC (docetaxel + doxorubicin + cyclophosphamide), and FEC-T (5-FU + epirubicin + cyclophosphamide), in which three cycles of FEC are followed by three cycles of docetaxel (28).

Regardless of adjuvant therapy choices for postmenopausal women with breast cancer, several recommendations can be made to improve the cardiovascular risk profile. The use of statins, beta-blockers, and/ or angiotensin-converting enzyme inhibitors and sulfonylureas or metformin to manage hyperlipidemia, hypertension, and diabetes, respectively, is appropriate. Cardiotoxicity is a rare complication of breast cancer treatment and the incidence and severity of cardiotoxicity are dependent on cumulative dose of anthracyline, the type and combination of drugs used, the presence of coexisting diseases, the way of radiotherapy and combination therapy in the treatment of locally advanced breast carcinoma (29). Adriamycin (doxorubicin) is one of the most effective chemotherapeutic agents against a variety of cancers, but its usefulness is seriously curtailed by the risk of developing heart failure (30).

Based on an emerging body of literature and the fact that cardiac disease can be clinically silent, it is recommended that lipid profile must be assessed in breast cancer patients, especially in postmenopausal patients, on regular basis. In conclusion, our results seemed to suggest a possibility that serum lipid levels could be considered as risk factors for breast cancer and associated CVD. Hence, dietary vitamin C supplementation, in postmenopausal breast cancer patients and adoption of a new combination of chemotherapeutic drugs, superior to FAC in its efficacy, with lower side effects may ensure a disease free survival after treatment, in our population. Small number of subjects used is the main limitation of our study, so a study on large cohort is required to confirm the application of our results.

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The survey of microRNA-196, microRNA-99a and estrogen receptor alpha expression levels in oligospermic infertile men

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Abstract

Estrogen receptors (ERs) mediates estrogen action in regulating at all levels of the hypothalamic-pituitary-testis axis in male. It has an essential role in male reproductive tract and spermatogenesis. In male mice estrogen receptor alpha (ERα) knock-out (ER koα), these mice were infertile and severe impairment in spermatogenesis, sperm motility and in vitro fertilizing potential was observed. Recently it has been reported that microRNA (miRNA) mir-99a and mir-196b were predicted to target ERα gene. miRNAs are small, endogenous, single stranded RNA molecules that regulate gene expression and have been implicated in various disease states. It has been proved that some miRNAs expression is tissue-specific and disease-specific, giving potential for identifying miRNAs as a diagnostic tool. About 60-75% of male infertility cases is idiopathic and their molecular mechanisms are unknown. Semen analysis and diagnostic tests are not accurate in this case. Therefore, taking the proper approach in treatment is difficult. In this study, changing in the expression levels of mir-99a,mir-196b and ERα expression levels were evaluated in oligospermic infertile patients (n=43) compared to control fertile subjects (n=43). After washing and separating sperms, total RNA was isolated and then cDNA was synthesized. The expression levels of mir-99a and mir-196b and ERα were evaluated by real time PCR method. mir-99a, mir-196b levels were significantly higher than those in normal controls($p< 0.0001$, $p=0.004$ respectively). Also, we have found that, ERα level significantly decreased in comparison with normal group ($p< 0.0001$). The increases of mir-99a, mir-196b and decreases of ERα expression in spermatozoa cells in oligospermic patients may be associated with the susceptibility and progression of infertility. The results of this study indicate that miRNA can be have a key role in spermatogenesis and might have a diagnostic and prognostic value in men infertility.

Key words: Has-mir-99a, hsa-mir-196b, microRNA, Male Infertility, ERalpha.

Introduction

Infertility is defined as a failure to conceive after more than one years of regular intercourse without contraception (1). Approximately 15% of the couples are infertile, about 45% of which is related to male factors. Oligospermia and asthenospermia are common causes of infertility in males, however, the molecular mechanisms causing these effect is not entirely clear. The causes are known in less than 35% of these cases, out of which is genetic disease with unknown molecular mechanisms. In oligospermia the number of spermatozoa is reduced and in asthenospermia abnormality in sperm motility is seen(2). About 60-75% of infertility cases are idiopathic with or without abnormal semen analysis(3). It was seen that in bulls, spermatozoa with normal motility and morphology, the fertility of some bulls was reduced which may be due to molecular defects in the sperm cells (4).

More than hundreds of genes are involved in spermatogenesis. Molecular and cellular integrity of sperm cells is important for fertilization any deletion or mutation in the sequence of genes and inappropriate gene expression causes disorder in spermatogenesis and fertility (5, 6). Estrogen has a positive impact on function of sperm by stimulating of capacitating and fertilizing ability (7), as well as it has a key role in modulating of the male

reproductive tract. Cellular signaling of estrogen is mediated through the estrogen receptors (ER) (8) that are present throughout the male reproductive tract and spermatozoa. ERα mediates estrogen action in regulating at all levels of the hypothalamic-pituitary-testis axis in male. ERα has an essential role in male fertility, it was proved that ER knockout (Erα-/-) mice become infertile (9).

ERα gene expression is regulated by small noncoding RNAs (microRNAs). Previous studies showed that mir-99a and mir-196b were predicted to target ERα gene (10, 11). MicroRNAs (miRNAs) were first detected in human spermatozoa by Ostermeier et al. they are abundant in spermatozoa(12) but their function in spermatogenesis and fertilization is unknown. miRNAs are small (18-25 nucleotides) noncoding regulatory RNAs which negatively regulate gene expression (13).They participate in designation of cell fate, embryonic development, and control of growth, differentiation, and death of cells (14). Number of miRNA is expressed in mouse male germ cells. miRNAs are involved in regulating of gene expression during mitotic, meiotic, and post-meiotic stages of spermatogenesis (15). Impaired biogenesis of miRNAs disrupts spermatogenesis and causes infertility in male mice (16).In the present study we investigated expression levels of mir-99a, mir-196b and their common target gene (ERα) and their correlation in oligospermic infertile and normospermic fertile men.

Material and methods

Study design

From infertile men (n=723) referred to the Tabriz Alzahra Infertility Center (mean age 27.5 years), which despite of continuous intercourse they had a background of infertility for more than two years,43 oligospermic infertile patients were selected. The written consent of the subjects was done according to the rules of medical ethics. Control samples (n=43), selected from normal volunteers, had a baby in the last two years and their semen analysis was normal. Two months before sampling, none of the control subjects nor patients treated with the drug, as well as they didn't have intercourse 3-5 days before sampling. This research was approved by the Ethics Committees of Tabriz medical University.

Exclusion criteria

The volunteers with infertile partner, infection in the genital tract, autoimmune disorders, reproductive tract abnormality, smoking, alcohol and drug consumption were excluded from the study.

Hormone detection

The blood was placed at 37 °C for 10 min to clot formation. The clot from the wall of the test tube was gently removed Then supernatant wascentrifugedat1000×g for 10 min at 4 °C. samples were assayed for follicle stimulating hormone (FSH), luteinizing hormone (LH), 17β-estradiol (estrogen) and testosterone using an enzyme-linked immunosorbent assay (ELISA) by commercial ELISA kit (AccuBind ELISA, Monobind, USA) according to the manufacturer's instructions.

Isolation of spermatozoa from seminal fluid

Semen samples were collected in a sterile container and incubated at 37 °C for 30 minutes to get the fluid then according to WHO guidelines, semen analysis was performed. Sperms were purified by Goodrich methods (17). In brief, the samples were washed two times in 1×PBS buffer solution, then somatic cells were absent in SCLB solution(0.1% SDS, 0.5% TX-100 in DEPC water). The cells were counted, if somatic cells were, present the process was repeated. Finally, the solution was frozen at –80 ºC.

RNA isolation

Total RNA was isolated using exiqon miRCURY RNA isolation kit (Exiqon, Denmark) according to the manufacturer's instructions. Quantity and quality of the isolated RNA was measuredbyNanodrop1000 (NanoDropND-1000 spectrophotometer; Thermo Fisher Scientific, Waltham, MA). Total RNAs were reversed to cDNA using LNA universal RT miRNA PCR kit (Exiqon, Denmark). Briefly, 20ng of total RNA was reverse transcribed. cDNA Synthesis was performed by thermal cycler (Eppendorf, Germany) with the following parameter value, 60 minutes at 42 ºC, 5 minutes at 95 ºC and immediately cool to 4 ºC until use.

Real-time PCR analysis

Quantitative real-time reverse transcriptase-PCR was carried out by using the Corbett Rotor-Gene 6000 Real-Time PCR system (Qiagen,

Germany). miRNAs quantification was performed using MiRCURY LNA™ Universal RT microRNA PCR system (Exiqon, Denmark). Mir-16 was used as the endogenous control miRNA. The relative expression level of ERα was measured by qPCR with primers (ERα: *5′-CCACATCAGTCACATGAGTAA-3′*and*5′-GTTCCATCAGCATCTACAG-3′*) using the QuantiTect SYBR Green PCR Kit (Qiagen, Germany). The expression levels were normalized to β-actin as housekeeping gene with the following primers (*5′-TGGACTTCGAGCAAGAGATG-3′*and*5′-GAAGGAAGGCTGGAAGAGTG-3′*). The reactions were performed in triplicate.

Statistical analysis

Statistical analysis was performed using SPSS software (version 18). The results were expressed as mean ± SD. Relative expression level of genes were calculated by using the 2^{DDCq} model (18).Unpaired Student's t-test was used to analyze the differences in gene expression between oligospermic and control group. Correlation analysis was performed using the spearman rank correlation test. In all analyzes P-value < 0.05 was considered as significant.

Results

Expression level of mir-99a, mir-196b and ERα in oligospermic and control group

We determined the expression levels of mir-99a, mir-196b and ERα in oligospermic and control group. By real-time quantitative RT-PCR analysis, we found that expression levels of mir-99a and mir-196b were much higher in oligospermic than control group (p<0.0001 and p=0.004, respectively; figure 1). Inversely, expression level of ERα was significantly lower in oligospermic than control group (p<0.0001; figure 1).

Correlation between expression levels of ERα and seminal plasma parameters

Correlation between expression levels of ERα and semen were analyzed using spearman's rank correlation test (table 1). Expression levels of ERα were strongly and positively correlated with those of sperm count, quick progressive, slow progressive and normal morphology (spearman's correlation coefficient; +0.863, +0.723, +0.875 and +0.642, respectively) and negatively correlated with immotile (spearman's correlation coefficient; -0.691).

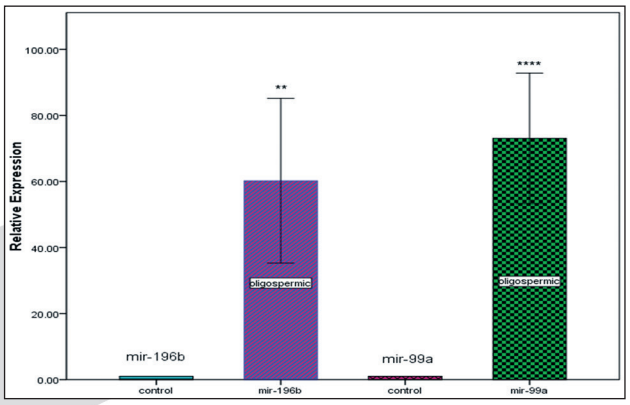

Figure 1. Relative expression levels of mir-196b and mir-99a in oligospermic and control group

Correlation between expression levels of miRNAs and seminal plasma parameters

Relationship between expression levels of miRNAs and semen parameters, such as volume, sperm count, quick progressive, slow progressive, non-progressive, immotile, normal morphology and pH, was performed using Spearman's rank correlation test (table 1). Expression levels of mir-196b were strongly and positively correlated with pH (spearman's correlation coefficient; 0.7295).

Correlation between hormones and ERα, mir-99a, mir-196b

Expression levels of miRNAs were not significantly correlated with hormones. Expression levels of ERα were strongly and positively correlated with estrogen(spearman's correlation coefficient; 0.7818) (table 2)

Discussion

Spermatogenesis is an intricate process of germ cell development that many genes are involved. Any defect in genes expression or their regulation disrupt spermatogenesis and cause infertility (19). miRNAs regulate gene expression by modification of special mRNA translation. Few studies have been conducted about miRNAs function in spermatogenesis and male fertility. In a study conducted on non-obstructive azoospermic infertile patients, significant change in miRNAs expression was seen compared to fertile control men (20). In that studyLian et al. showed that miRNAs have a regula-

Table 1. Correlation analysis between ERα, mir-99a, mir-196b and seminal plasma parameters

variable	mir-99a	mir-196b	ERα
volume	0.06791[a]	0.4770	-0.092
	0.8521[b]	0.1666	0.799
Sperm count	0.01824	0.1220	0.863*
	0.9601	0.7372	0.001
Quick progressive	0.4975	0.2138	0.723*
	0.1435	0.5530	0.018
Slow progressive	0.2601	0.02174	0.875*
	0.4679	0.9525	0.0009
Non-progressive	0.3525	0.3472	0.551
	0.3179	0.3256	0.098
Immotile	-0.4390	-0.1835	-0.691*
	0.2043	0.6119	0.026
Normal morphology	0.4280	0.4640	0.642*
	0.2173	0.1767	0.045
pH	0.2848	0.7295*	0.345
	0.4250	0.0166	0.328

[a] Spearman correlation coefficient
[b] P, spearman's rank correlation test
** P<0.05 is consider significant*

Table 2. Correlation analysis between ERα, mir-99a, mir-196b and hormones

variable	mir-99a	mir-196b	ERα
FSH	-0.5152[a]	0.1033	0.2364
	0.1276[b]	0.7763	0.5109
LH	-0.3697	0.1398	0.3455
	0.2931	0.7001	0.3282
E2	-0.2970	-0.3587	0.7818*
	0.4047	0.3088	0.0075
test	0.2000	0.2918	-0.1879
	0.5796	0.4133	0.6032

[a] Spearman correlation coefficient
[b] P, spearman's rank correlation test
** P<0.05 is consider significant*

tory role in spermatogenesis. In this study we investigated mir-99a, mir-196b and their common target ERα gene expression in oligospermic infertile patients compared with normospermic fertile control by real-time PCR methods. Our result showed that mir-99a in oligospermic was significantly over expressed compared to control group. We also demonstrated that high mir-99a expression was associated with significant decreases in ERα gene expression level in oligospermic group. It was proved that mir-99a was predicted to target ERα, which has been implicated in ERα regulation(10, 21).In the present study we have shown that expression level of mir-196b was increased unlike ERα expression, which was decreased. Guarducciet al. showed that ERα promoter polymorphism were inversely associated with sperm count (22). As our results indicated inhibition of germ cell proliferation by reducing the expression of ERα gene bymir-196b is possible. In the male reproductive tract, there are higher levels of ERα in the efferent ductules (region of the male tract) than female reproductive system, it occupy one third of epididymis. It shows the importance of ERαin the male reproductive system and fertility. ERα has a key role in the regulation of fluid reabsorption, and in the epididymis the receptor is re-

sponsible for keeping fluid osmolality and pH(23). In our study reduced ERα expression was associated with little change in semen pH. Gunawanet al. showed that a polymorphism in the coding region of ERα in exon 1 was related to sperm motility (24). Recent findings are consistent with our results. We proved that expression level of ERα in oligospermic significantly was down regulated compared to control group, as well as motility and pH in seminal fluid of oligospermic was lower than those in control. Our data showed that Spermatozoa with normal morphology decreased in oligospermic group compared to control group as well as our results showed that significant positive correlation with the expression of ERα and morphology. This is consistent with recent findings of Josepha and collegues. They proved thatin ERαKO mice sperm maturation and capacity to fertilize was destroyed that contributes to infertility (25).ERα play an important role in modulation of sperm metabolism (26), disruption in its performance, reduced values for sperm density, sperm motility, and percentage of sperm with normal a unique property of GS (germline stem cell) morphology (27).ERα in the acrosome of the spermatozoa is more than in other sections, acrosome contains lytic which Puncture the outer coat of the egg and allow the infiltration of sperm (28). Acrosome dysfunction impairs oocyte fertilization and cause of male infertility (29). Oligospermic patients have a high frequency of defective sperm–zona pellucida (outer coat of the egg) interaction (30, 31)according to our finding decrease in ERα may cause acrosome dysfunction.

It is possible that miRNA interfere in spermatogenesis through other genes. Possible target of mir-99a are SLC2A and DICER1.SLC2A (solute carrier family 2) is belong to glucose transporter (GLUT) family. It is expressed in the testes and sperm. It was showed that altered SLC2A expression may be responsible for the decreased spermatogenesis, sperm maturation, and fertilization in the male mice.(32, 33).DICER1 (RNAse III endonuclease) is essential for the biogenesis of microRNAs (miRNAs). DICER1 is essential for differentiation of the male germline and spermatogenesis.(34, 35). AKT1 is a target for mir-196b. AKT1 is a serin/theronin kinas enzyme that it has been proved to be the mediator of cellular growth, proliferation, survival, and metabolism in various cell types (36). Kim et al. showed that in Akt1-/- male mice apoptotic sperm in null mice were more than wild-type mice, and sperm motility and concentration were significantly lower in the null sperm (32).

In conclusion, we have defined efficacy of mir-99a, mir-196b and ERα in oligospermic infertile patients. Our results obtain more information about the molecular mechanism of infertility, and their possible regulatory role in spermatogenesis and fertilization.

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Skeletal muscle peroxisome proliferator-activated receptors (PPARs) and PPAR-γ coactivator (PGC)-1α proteins are differentially expressed in response to Opuntia humifusa supplementation and acute exercise in rats

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Abstract

Objective: We examined the effects of *Opuntia Humifusa* (O. Humifusa) supplementation and acute exercise on skeletal muscle PPARs and PGC-1α protein expression in rats.

Methods: Sprague-Dawley male rats were fed either a high-fat diet (HS: sedentary group, HE: exercised group, both n=8) or a 5% of O. Humifusa supplemented diet (OS: sedentary group, OE: exercised group, both n=8) for 4 weeks. At the end of the experimental period, the rats of exercised groups swam for 60 min.

Results: Muscle PPAR-δ levels of the OS was significantly higher than that of the HS ($p<.05$). Also, the PGC-1α levels of the OS tended to be higher than that of the HS. However, PPAR-γ expression in exercised groups was significantly decreased compared to that of the sedentary groups ($p<.01$).

Conclusion: The findings suggest that PGC-1α, PPARs protein expression in skeletal muscle may be an important adaptive mechanism in response to O. humifusa supplementation and acute exercise in rats.

Key words: Opuntia Humifusa, exercise, peroxisome proliferator-activated receptors, peroxisome-proliferator-activated receptor-γ coactivator-1α, skeletal muscle.

Introduction

During aerobic exercise, lipid and glucose are oxidized in the mitochondria of skeletal muscle as main energy substrates. In addition, lipid oxidation and insulin sensitivity is regulated by peroxisome-proliferator-activated receptor-γ coactivator-1 (PGC-1α) [1], peroxisome proliferator-activated receptors (PPARs) [2], sterol regulating element binding protein-1c (SREBP-1c) [3], and the forkhead type transcription factor (FKHR) [4]. Of these, PGC-1α, a transcriptional co-activator, is abundant in skeletal muscle [5] and plays an important role in the regulation of mitochondrial biogenesis and insulin sensitivity [6]. Furthermore, increased cytosolic Ca^{2+} concentration affects PGC-1α protein expression and mitochondrial biogenesis in vitro [7].

In addition to the physiological role of the PGC-1α gene, the PPAR-δ gene, a member of subfamily 1 of the superfamily of nuclear receptors [8], is also involved in mitochondrial biogenesis including lipid catabolism in skeletal muscle during exercise [9]. In contrast to PPAR-δ, PPAR-γ is abundant in adipose tissue and participates in the improvement of carbohydrate metabolism and lipid storage [10]. In the study, acute exercise was associated with increased skeletal muscle PGC-1α and PPAR-δ mRNA expression immediately and 1-4 h after exercise, respectively. Also, skeletal muscle PPAR-γ mRNA was slightly decreased compared to the pre-exercise state. However, whether PGC-1α and PPARs are changed in transcriptional level remains unknown.

Opuntia humifusa (also known as Eastern Prickly Pear, Devil's Tongue, and Low Prickly Pear) has therapeutic value by virtue of its antioxidative and anti-inflammatory effects [11] in addition to its hypoglycemic and hypolipidemic

effects [12]. Quercetin present in O. humifusa contains a number of phenolic hydroxyl groups that have strong antioxidant activity [13,14]. A recent study reported that Opuntia Humifusa (O. humifusa) is enriched in a variety of minerals including Ca^{2+} that increases bone mineral density in growing rats [15]. Interestingly, with respect to relationship of Ca^{2+} and mitochondrial biogenesis, an increased cytosolic Ca^{2+} concentration also induces stimulation of skeletal muscle mitochondrial biogenesis [7] and leads to the activation of PGC-1α through the induction of CaMK II and p38 mitogen-activated protein kinase [16].

The present study aimed to investigate the effects of O. humifusa on the expressions of PGC-1α and PPARs proteins in rat skeletal muscle after acute swimming exercise.

Materials and methods

Experimental animals and group classification

Thirty-two, 6-week-old Sprague-Dawley rats weighing 230-250 g were purchased from Samtaco Bio Korea (Hwaseong, South Korea). The rats were randomly divided into four groups (n=8 per group): high-fat diet sedentary group (HS), high-fat diet acute exercised group (HE), high-fat + O. humifusa supplemented sedentary group (OS), and high-fat + O. humifusa supplemented acute exercised group (OE). All rats were given free access to tap water and diet for 4 weeks, and were housed in groups of two per cage under controlled temperature (23±1°C) and relative humidity (50±5%). The light/dark cycle was automatically controlled in alternating 12-h periods, with lighting beginning at 08:00. This study adhered to the *Guide for the Care and Use of Laboratory Animals* developed by the Institute of Laboratory Animal Resources of the United States National Research Council and was approved by the Institutional Animal Care and Use Committee of Sunmoon University in Asan, Republic of Korea.

Preparation of O. humifusa

Crude extract of O. Humifusa was kindly gifted from Foodsam Co. (Cheonan, Korea). Briefly, the stem of O. humifusa was obtained from the Asan area. It was washed and blended using a model HMF-3150S blender (Hanil, Korea). After blending, the extract was added to a 20% (w/v) sugar solution containing Saccaromyces cerevisiae (10^9/ml). After 2 days, it was fermented with addition of lactobacillus acidiphillus (10^9/ml) during incubation at 35-45°C for 10 days. The contents of crude extract were reported in a previous study [17]. The results of composition were shown at Table 1. Aliquots of the fermented O. humifusa extract were orally administered (2 mL/day) to the groups of rats described below. Other groups, also described below, received 2 mL/day of tap water.

Diets and exercise

The high fat diet was composed of 20% protein, 35% carbohydrate, and 35% fat, which was modified from a previous study [18]. During the experimental period, the diets were prepared fresh in 3–5 day batches and stored at 4 °C to maintain freshness. Food intake and body weight were measured daily and weekly, respectively. The food efficiency ratio (FER) was calculated as the total weight gained divided by the total food intake for the experimental period. At the end of the experimental period, the sedentary rats were anesthetized with diethyl ether after fasting for 12 h. At the same time, the rats of the exercised groups were made to swim for 60 min (3% load/body weight). All rats were sacrificed and blood samples were taken from the left ventricle. Serum was obtained by centrifuging the blood at 700× *g* for 20 min at 4 °C. Serum and obtained hindlimb muscle samples were stored at −70 °C until further analysis.

Biochemical analysis

Serum glucose, total cholesterol (TC), triglyceride (TG), and high density lipoprotein-cholesterol (HDLC) levels were analyzed using commercial enzymatic kits (Asan Pharmaceutical, Yongin, South Korea).

Western blot analysis

Hindlimb muscles were homogenized on ice with a polytron homogenizer in 19 volumes of 20 mmol/L Tris-HCl buffer (pH 7.5) containing 5 mmol/L EDTA, 2 mmol/L phenylmethanesulfonylfluoride, and protease inhibitor cocktail (Sigma-Aldrich, St Louis, MO). The homogenates were centrifuged at 1,200 × g for 10 min and the supernatant was collected and

recentrifuged at 10,000 × g for 10 min. The resulting supernatants were used for analyses of PGC-1α and PPARs protein expressions. Protein concentrations were determined by the use of Bradford reagent (Bio-Rad Laboratories, Hercules, CA) with bovine serum albumin as the standard. All protein extraction procedures were conducted at 4oC. An aliqout of tissue extract containing 20 μg protein was mixed with an equal volume of Laemmli buffer, heated at 100oC in a heating block for 5 min, and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis along with a mixture of molecular-weight standards (Bio-Rad Laboratories). After electrophoresis, the proteins were transferred to a polyvinylidene fluoride membrane (Bio-Rad Laboratories) in a Mini Transfer-Blot electrophoretic Transfer cell (Bio-Rad Laboratories). After treating with blocking buffer consisting of phosphate buffered saline (PBS) containing 10% skim milk for 90 min at room temperature, each membrane was incubated with primary polyclonal antibodies for 2 h at room temperature. The antibodies were anti-PGC-1α (Santa Cruz Biotechnology, Santa Cruz, CA), anti-PPAR-γ (Santa Cruz Biotechnology), and anti-PPAR-δ (Santa Cruz Biotechnology). Each membrane was then incubated with horseradish peroxidase-conjugated anti-goat IgG or anti-rabbit IgG secondary antibody (Santa Cruz Biotechnology), as appropriate, for 1 h at room temperature. The target proteins were detected by an enhanced chemiluminescent kit (GE, Buckinghamshire, UK). The films were photographed and the protein bands of interest were quantified with band analyzer software (Bio-Rad Laboratories).

Statistical analyses

All data were analyzed using SPSS software version 16.0 for Windows (SPSS, Chicago, IL). Data are expressed as the mean ± SEM and values were analyzed by one-way analysis of variance (ANOVA) followed by Scheffe's test. Significan-

Table 1. The chemical and mineral compositions in fermented liquid of O. Humifusa

	Contents
Energy (kcal/ 100g)	86.21
Protein (%)	0.92
Lipid (%)	0.12
Carbohydrate (%)	20.34
Ca (mg/100g)	1,800
Fe (mg/100g)	21
Mg (mg/100g)	497
Zn (mg/100g)	10

Table 2. Changes of body weight, fat weight and food efficiency ratio

	HS	HE	OS	OE
Initial body weight (g)	204.8±2.72	204.9±3.27	204.9±2.77	205.0±2.70
Final body weight (g)	317.2±10.74	316.3±6.77	305.2±4.22	298.6±4.50
Food efficiency ratio	0.31±0.02	0.33±0.02	0.33±0.03	0.32±0.02
Abdominal fat pad (g)	4.9±0.57	5.3±0.37	4.5±0.4	4.6±0.39
Epididymal fat pad (g)	4.5±0.42	5.4±0.33	4.2±0.18	4.3±0.26

Data are mean ± S.E.M. HS: high fat diet sedentary group, HE: high fat diet acute exercise group, OS: O. humifusa supplemented sedentary group, OE: O. humifusa supplemented acute exercise group

Table 3. Changes of serum components

	HS	HE	OS	OE
Glucose (mg/dl)	155.2±1.56	142.1±1.69	120.4±8.12**	139.6±7.53
Triglyceride (mg/dl)	205.6±7.78	194.8±7.05	182.5±4.99	189.2±4.41
TC (mg/dl)	104.8±3.70	112.8±5.11	86.1±2.99*	94.2±3.31
HDLC (mg/dl)	29.1±1.40	29.7±0.65	31.9±2.10	29.6±1.01

*Data are mean ± S.E.M. *p<.05, **p<0.01: compared with HS. HS: high fat diet sedentary group, HE: high fat diet acute exercise group, OS: O. humifusa supplemented sedentary group, OE: O. humifusa supplemented acute exercise group*

ce was defined as α=0.05.

Results

As shown in Tables 1 and 2, the body weight, fat weight and FER of the OS and OE groups were not changed significantly compared to the HS and HE groups, respectively. However, blood glucose and TC level of OS rats were significantly decreased, compared to HS rats (Table 3).

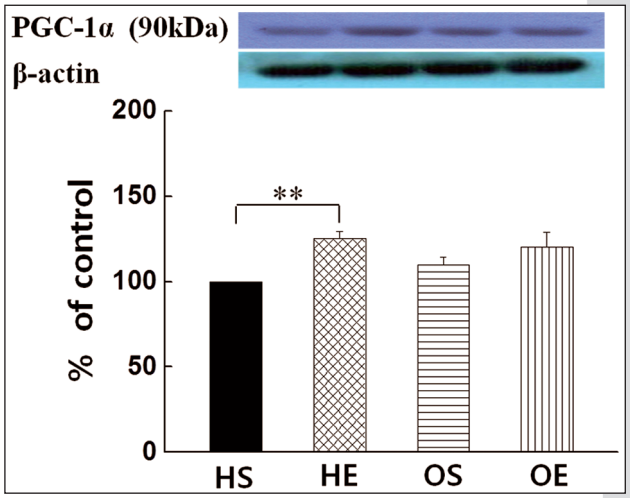

*Figure 1. The changes of PGC-1α protein expression with O. humifusa supplementation and acute exercise. HS: high fat diet sedentary group, HE: high fat diet acute exercise group, OS: O. humifusa supplemented sedentary group, OE: O. humifusa supplemented acute exercise group. Values are mean ± SEM. **p<0.01*

In the present study, as shown in Figure 1 and Figure 2, O. humifusa supplemented groups showed increased (but non-significant) PGC-1α expression in rat skeletal muscle. In addition, muscle PPAR-γ expression of the OS group of rats was significantly increased compared to HS rats. In addition, we found that PGC-1α and PPAR-δ protein expression was significantly or slightly increased, and PPAR-γ protein expression was significantly decreased after acute exercise.

Discusssion

O. humifusa is a traditional health herb [19,20] that contains a lot of Ca and Mg contents as shown at Table 1. In the present study, we investigated the effects of O. humifusa supplementation on skeletal muscle expression of PPARs and PGC-1α proteins in rats during resting and following acute exercise.

Our results are compatible with the prior demonstration of the significantly decreased blood glucose and TC level of O. humifusa supplemented diabetic rats [12]. These hypoglycemic and hypolipidemic effects of O. humifusa are attributed to elevated antioxidant activity. O. humifusa is enriched in antioxidants including quercetin [11] Glycoprotein isolated from Opuntia ficus-indica, another member of the family containing O. humifusa, has potential scavenging capacity with hypoglycemic and hypolipidemic effects [21].

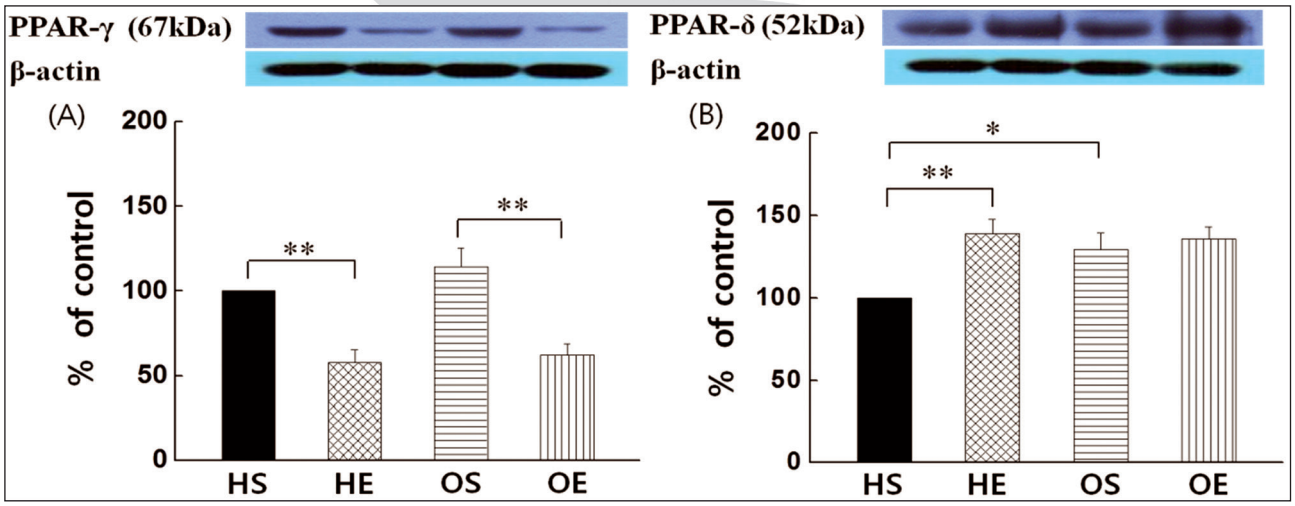

*Figure 2. The Changes of PPAR-γ (A) and PPAR-δ (B) protein expression with O. humifusa supplementation and acute exercise. HS: high fat diet sedentary group, HE: high fat diet acute exercise group, OS: O. humifusa supplemented sedentary group, OE: O. humifusa supplemented acute exercise group. Values are mean ± SEM. *p<0.05, **p<0.01*

With respect to exercise, muscle PPAR-γ expressions of rats in the HE and OE groups were significantly decreased compared to HS and OS rats, respectively. Contrary to PPAR-γ expression, muscle PPAR-δ expression of HE was significantly increased compared to HS. In addition, muscle PPAR-δ expression of OS was significantly increased compared to HS. However, there was no significant difference between Os and OE. O. humifusa is enriched in a variety of minerals including Ca and Mg, which increase bone mineral density in growing male rats [15]. In addition, intracellular Ca has a crucial role in mitochondrial biogenesis in muscle [7]. In one study, skeletal muscle PGC-1α expression in rats was significantly increased after exposure to 3.5 mM caffeine for 6 h, which elevated cytosolic Ca concentration [16]. This result indicates that an increased intracellular Ca concentration stimulates mitochondrial biogenesis including PGC-1α, nuclear respiratory factor (NRF)-1 and NRF-2. O. humifusa supplementation may affect the expression of PGC-1α and PPARs proteins in muscle in a variable fashion according to the method used, period or analysis, or composition of the diet.

With respect to acute exercise, it has been reported that gene expressions of metabolic transcriptional co-activators and transcriptional factors are differentially expressed after exercise. Specifically, PGC-1α mRNA was increased immediately after exercise and PPAR-δ mRNA was increased 4 h after exercise, while PPAR-γ mRNA was not appreciably changed, although a decreasing trend after exercise was evident [4]. However, protein expression of muscle PGC-1α, PPAR-γ, and PPAR-δ after acute exercise has not hitherto been clear.

Our results indicate that PPAR-δ protein mimics the role of PGC-1α and PPAR-γ protein expression, contrary to PGC-1α in skeletal muscle after acute exercise. In fact, PGC-1α has a crucial role for mitochondrial biogenesis [23] and PPAR-δ is related to lipid catabolism [10]. However, PPAR-γ expression is closely related to regulation of whole body insulin sensitivity [24]. In the present study, the exercised rats swam for 60 min, which stimulated lipid catabolism and inhibited insulin secretion during the endurance exercise. Therefore, we suggest that endurance exercise induces increased muscle PGC-1α and PPAR-δ protein expression, and decreases PPAR-γ protein expression of the exercised rats.

Acknowledgements

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Nutritional status on admission and clinical outcomes of critically ill patients

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Abstract

Background: The prevalence of malnutrition is a common problem in hospitalized patients, especially in critically ill patients.

The purpose of this study was to correlate the nutritional status of intensive care unit (ICU) patients with their morbidity, mortality, length of ventilator stay and length of ICU stay.

Methods: We prospectively studied 432 patients ≥ 18 years that stayed more than 4 days in the ICU of QSUT.

Univariate and multivariate logistic regression analysis were used to identify the relation between malnutrition and outcome.

Results: The prevalence of malnutrition in ICU admission was 63.6%. Malnutrition, as analyzed by a multivariate logistic regression model, is an independent risk factor on higher complications, higher infectious complications, increased mortality, longer stay in the ventilator and longer ICU stay.

Conclusions*:* Malnutrition affects negatively the outcome of ICU patients.

Key words: Critically ill, malnutrition, morbidity, mortality, length of ICU stay.

Introduction

Malnutrition is a persistent problem in hospitals and intensive care units (ICUs) worldwide. Upon admission to hospital, about 15–50% patients are under- or malnourished. [1-7]

Malnutrition has been described as the imbalance between intake and requirement which results in altered metabolism, impaired function and loss of body mass [8] or as a state of nutrition in which a deficiency or imbalance of energy, protein, and other nutrients causes measurable adverse effects on tissue and/or body form. [9]

In developed countries the main cause of malnutrition is disease. Any disorder, whether chronic or acute, has the potential to result in or aggravate malnutrition. The reasons for developing malnutrition in sickness are multifactorial, but decreased nutritional intake, increased energy and protein requirements, increased losses together with inflammation probably play the central role.

Critically ill patients quickly develop malnutrition or aggravate a preexisting malnutrition because of the inflammatory response, metabolic stress and bed rest, which all cause catabolism. [10,11]

Malnutrition has been identified as affecting patient outcome. The acute phase response in critical illness induces catabolism, through a cascade of reactions, which, if left untreated, accelerates the precipitation or worsening of malnutrition and is associated with death. [12]

The purpose of this study was to correlate the nutritional status of ICU patients with their morbidity, mortality, length of ventilator stay and length of ICU stay.

Material and methods

We retrospectively studied 432 patients ≥ 18 years that stayed more than 4 days in the ICU of University Hospital Center of Tirana "Mother Theresa" between 2010 and 2012.

Nutrition risk screening

Nutritional status on admission was assessed according to Nutritional Risk Screening 2002 [13]. It contains one scale to examine nutritional status (0-3 points) and one scale to assess potential changes in stress metabolism (0-3 points.) A total score > 3 indicates that nutrition support should be initiated.

Patients were divided into well-nourished and malnourished groups, according to their nutritional status (NRS 2002 < 3, and NRS 2002 ≥ 3, respectively).

Demographic and medical information

Demographic and medical information including sex, age, date of ICU admission, ICU discharge, mechanical ventilation, ICU diagnosis, Acute Physiology and Chronic Health Evaluation (APACHE II) prognosis score [14] were collected.

Complications, mortality, length of ventilator stay and length of ICU stay

The patients' charts were reviewed searching for complications, infections, duration of ICU stay and length of ventilator stay, and ICU mortality. Infectious complications were defined as sepsis or systemic inflammatory response syndrome [15], pneumonia, urinary tract infection, central venous catheter sepsis, and wound infection. Other complications were: post-operative, metabolic disorders and organ's failure (by SOFA) [16]. The duration of time in the ICU was determined from the day of admission in the ICU to the moment of discharge or death.

Statistical analyses

Data are presented as the mean ± SD (standard deviation) for numerical variables, number (n) or percentage (%) for categorical variables. Multiple regression analysis was used to analyze the effect of malnutrition on length of ICU stay, length of ventilator stay, total complications, infectious complications and on mortality.

The correlation between nutritional status and clinical outcome was assessed and adjusted for confounders. Categorical data were analyzed using the Ch^2 test. The relative risks (RR) between the risk factor variable (presence of malnutrition) and the outcome variables (morbidity, mortality and length of ventilator stay and ICU stay) were calculated with their confidence intervals (CI). Those variables considered risk factors for complications and mortality (by the univariate analysis) were entered into the multivariate logistic regression model, to calculate the odds ratio (OR) and its confidence interval. Statistical significance was considered at the level of $p < 0.05$ for descriptive statistics and $p < 0.10$ was considered to be statistically significant for the logistic regression analyses. SPSS statistical package version 15 was used to analyze the data.

Results

A total of 432 patients were studied. The mean age was 62.96 ± 14.5 years old with 60.4% (n = 261) being male (figure 1). According to NRS 2002 the prevalence of malnutrition at the time of ICU admission was 63.6% (65.81% in the surgical patients and 57.98% in the medical patients, p = 0.16). The mean APACHE II score was 18.07 (± 5.54). ICU length of stay was 9.3 days (± 6.4), (figure 2); Mechanical ventilation lasted 2.7 days (± 4.1). ICU mortality was 28.7%. Cancer was present in 22.7% of the cases. 326 patients (75.46%) were post operative.

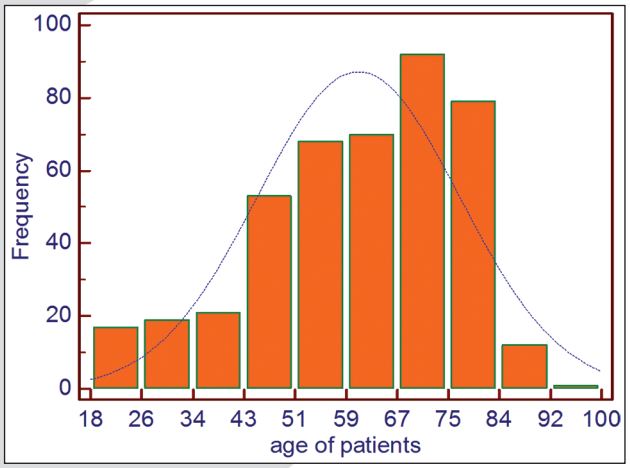

Figure 1. Histogram of distribution of patients according to the age (years)

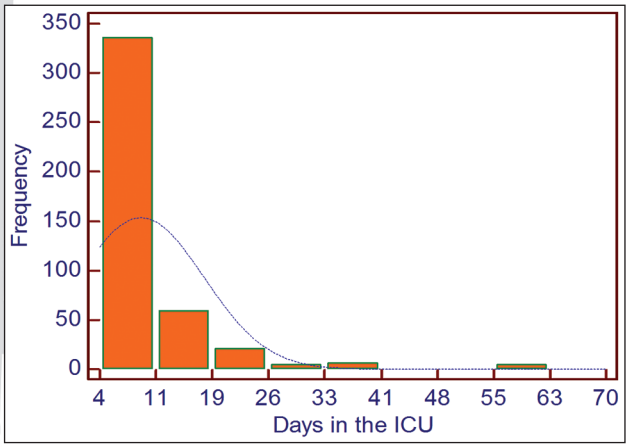

Figure 2. Histogram of distribution of patients according to the length of stay in the ICU

The overall incidence of complications was 53.94% (n = 233), but a significantly higher percentage was present in the malnourished patients. The incidence of complications in the malnourished was 68.3% vs. 28.6% in the well nourished

Table1. Outcome of the patients according to the nutritional status

	NRS 2002 < 3	NRS 2002 ≥ 3	P
Patients with complications (n/%)	45 (28.6%)	188 (68.3%)	< 0.001
Mortality (n/%)	33 (21.0%)	91 (33.0%)	0.01
Patients with infections (n/%)	35 (22.2%)	108 (39.2%)	0.0005
Length of stay in the ICU (mean ± SD)	7.31 ± 5.23	10.5 ± 9.35	< 0.0001
Length of stay on ventilator (mean ± SD)	1.62 ± 4.05	2.49 ± 4.48	0.04

Table 2. Multiple logistic regression model for risk factors of complications and mortality

Risk factors	Complications		Mortality	
	OR	95% CI	OR	95% CI
Malnutrition	5.37	3.50-8.26	1.85	1.17 - 2.94
APACHE II ≥ 15	2.31	1.55-3.44	3.56	2.15-5.90
Presence of infections	12.6	7.20-22.0	1.64	1.06-2.52
Mechanical ventilation	1.14	1.07-1.21	1.90	1.38-2.62
Age above 60 years	1.44	0.98-2.12	1.82	1.17-2.82

**p < 0.05*

patients [Odds ratio (OR): 5.3, 95% confidence interval (CI): 3.501 - 8.261; p = 0.0000], (table 1).

The incidence of infectious complications was 33.1% (n = 143). In the malnourished the incidence of infectious complications was 39.2% vs. 22.2% in the well nourished patients, OR = 2.2, 95% CI: 1.44 - 3.52; p = 0.0003.

Mortality in the malnourished patients was 33.0% vs. 21.0% in the well nourished (OR = 1.8, 95% CI: 1.17 - 2.94; p = 0.008), (figure 3).

Length of ventilator stay was shorter in the well-nourished patients. Malnourished patients stayed in the mechanical ventilation 2.49 ± 4.48 days vs. 1.6 ± 4.05 days in the well nourished patients, (figure 4).

Malnourished patients stayed in the ICU for 10.55 ± 9.35 days vs. 7.31 ± 5.23 days in the well nourished, (figure 5).

Malnutrition, as analyzed by a multivariate logistic regression model, is an independent risk factor on higher complications: F = 73.96, P < 0.001, higher infectious complications: F = 13.35, P < 0.001, increased mortality: F = 7.2, P = 0.008, longer stay in the ventilator: F = 4.03, P = 0.04 and longer ICU stay F: = 15.52, P < 0.001.

Other risk factors, such as the presence of cancer, APACHE II score ≥ 15, presence of infection, mechanical ventilation and age above 60 years old were associated with complications and mortality, by the univariate analysis. Therefore, all of these variables were entered in the multiple logistic regression model. Those, which were statistically different, can be seen in Table 2.

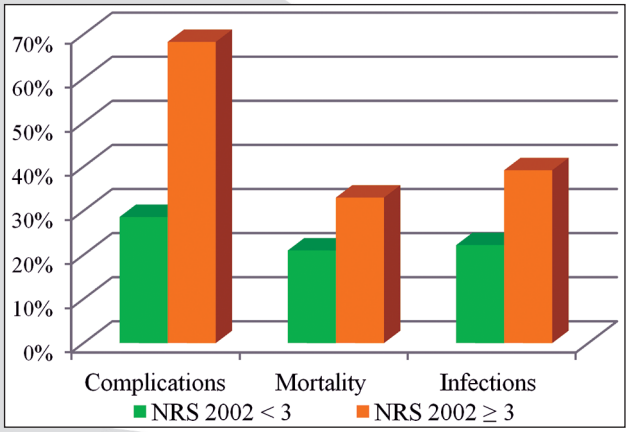

Figure 3. Incidence of complications, mortality and infections according to the nutritional status

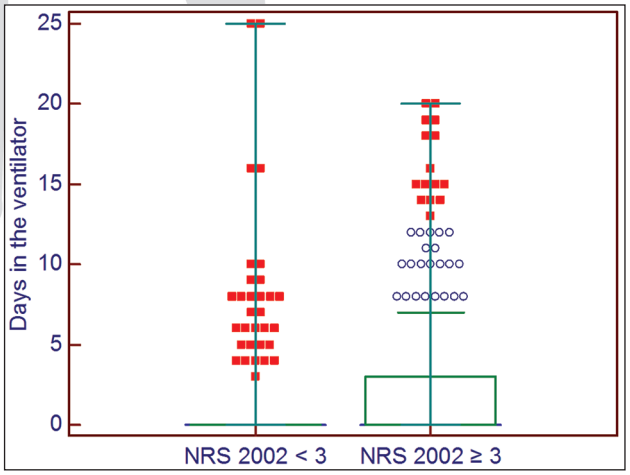

Figure 4. Length of ventilator stay according to the nutritional status

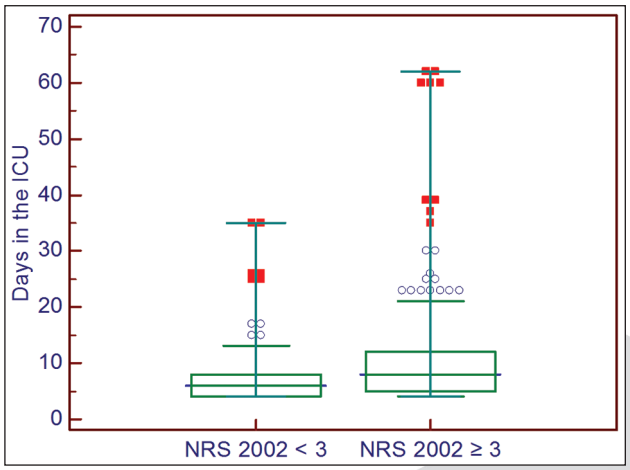

Figure 5. Length of intensive care stay according to the nutritional status

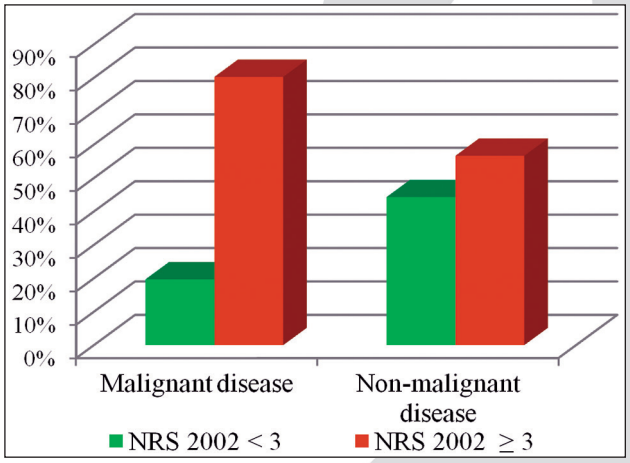

Figure 6. Prevalence of malnutrition according to t he presence of malignant disease

Patients with malnutrition had a higher relative risk for infectious complications compared with them without malnutrition, RR = 2.7, 95% CI (2.0 – 3.7), p < 0.001; and a higher relative risk for mortality, RR = 1.57, 95% CI: (1.11– 2.22), p = 0.01.

22.7% of the patients (n=98) were with malignant disease, from them 81 patients (82.65%) were with malnutrition on admission in the ICU (figure 6). Presence of malignancy, as analyzed by logistic regression model, is an independent risk factor on malnutrition: OR=3.43; 95% CI 1.95 - 6.05; p=0.00001.

Average age was significantly higher in those with malnutrition compared to those without (63.66 ± 15.26 and 56.23 ± 16.76, respectively, p < 0.0001).

69.4% of the malnourished patients were above 50 years old.

Age > 50 years showed a higher risk for the presence of the malnutrition, (RR = 1.87, 95% CI: 0.91 - 2.20; p = 0.002).

Presence of APACHE II score ≥ 15, as analyzed by logistic regression model, is an independent risk factor on malnutrition: OR = 3.23; 95% CI 2.14 – 4.87; p < 0.0001.

Despite the high prevalence of malnutrition at admission, nutritional therapy was prescribed to 51% of the patients. Enteral nutrition was used by 2% of all the patients, parenteral nutrition by 40%, mixed nutrition enteral-parenteral by 3% and oral nutrition by 6% of the patients.

Discussions

The prevalence of 63.6% malnutrition in medical and surgical patients staying for more than four days in intensive care unit confirms the severity of this problem. A recent review of the world literature found that in 20 studies since 1990 the mean malnutrition rate in the hospital was 41.7% [17].

In another study the prevalence of malnutrition in the patients of the intensive care unit was as high as 47.6% on admission, using NRS 2002 method [18].

Malnutrition is a consequence of several risk factors, of which the disease per se is one of the most important. The disease often leads patients to have negative net nutrient intakes, i.e. nutrient intake less than their requirements [19, 20].

In our study results that a patient group particularly predisposed to malnutrition is the elderly. In another review (35 studies, n = 8596) up to 74% of elderly hospitalized patients were undernourished or malnourished on admission [21], prevalence of malnutrition was 23 ± 0.5% (mean ± SE, range 1-74%). These findings are of serious concern since the undernourished elderly are known to have longer periods of illness, longer duration of hospital stay, higher infection rates, delayed wound healing, reduced appetite, and increased mortality [22].

The incidence of malnutrition in critically ill is particularly high in at risk patients [23]. In our study we found that APACHE II score above 15 is an independent risk factor on malnutrition.

Malnutrition is frequent in the sick elderly [24-26], in patients with malignant disease [27].

In our study we find the malignant disease a risk factor for malnutrition. 82.65% of the patients with malignant disease were with malnutrition on admission in the ICU.

Many patients admitted in emergency may have been suffering an illness and have had poor nutrition before admission to intensive care. In our study we did not find a correlation between emergency or elective patients and malnutrition.

Impaired immune function, delayed wound healing, and convalescence from illness and decreased functional status are the main contributors for the enhanced morbidity in malnutrition.

Previous studies have shown the impact of nutritional status on morbidity, mortality, LOS. ICU patients suffering from under-nutrition with a limited nutrition reserve have a poorer outcome [28].

Similarly to these studies, we were able to demonstrate that malnourished patients had significantly higher incidence of complications, increased mortality, longer ventilator and ICU stay. We used multiple logistic regression as a tool to assess the role of malnutrition alone in the patient's outcome, knowing that other variables could represent a source of bias [29]. By using this, we were able to show that malnutrition was indeed an independent predictor of outcome. In our study 314 patients (72.68%) were post operative, and was confirmed that malnutrition in surgical patients is associated with a poorer clinical outcome compared with well-nourished.

Other studies had shown that hospital malnutrition has been associated with an increased risk of complications, particularly in surgical patients [30, 31].

Unfortunately, disease and nutrition interact whereby the disease may cause secondary malnutrition or malnutrition may adversely influence underlying disease [20]. This makes it difficult to conclude that malnutrition alone leads to the patient's worst outcome [32].

Giner showed that malnourished patients in an intensive care unit have a poorer prognosis and survival [28].

Due to the increased morbidity, malnourished patients experience a significantly prolonged treatment duration and length of hospital stay. Two recent studies analyzing data from more than 1270 patients from two university hospitals in Geneva and Berlin demonstrated a close relationship between the degree of malnutrition and the length of hospital stay [33]. This clearly indicates that the definition and or criteria of malnutrition influence the associations between malnutrition and clinical outcome.

ICU mortality was also significantly influenced by the nutritional status of the patients. Malnutrition was considered an independent risk factor, witch significantly contributed to mortality.

Conclusions

We conclude that malnutrition is a common problem in critically ill patients and is associated with a poor outcome, increased morbidity and mortality.

Since it has been demonstrated that proper nutritional care can reduce the prevalence of hospital malnutrition and complications related with him, nutritional assessment should be routinely performed at admission in an attempt to reduce nutrition-related complications. Disease-related malnutrition can be treated, and nutrition-related complications, such as infections, length of ventilator and ICU stay, and mortality, can be improved.

Unfortunately, only a fraction of malnourished patients receives nutritional therapy due to a lack of awareness of the malnutrition- related adverse effects. Improved education of medical staff is needed and screening for malnutrition is mandatory.

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Left renal atrophy in case of splenomegaly

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Abstract

Background: We tried to understand whether or not there is a difference according to renal atrophy between the left and right sides in human body.

Methods: All patients applying to the Hematology Service were studied.

Results: The study included 2.417 cases (1.248 females). The mean ages were 47.3 versus 50.7 years in females and males, respectively (p<0.000). There were 33 cases (1.3%) with the left renal atrophy against five cases (0.2%) with the right (p<0.001). The left renal atrophy cases have splenomegaly in 51.5%, thalassemia minors in 30.3%, sickle cell diseases (SCDs) in 27.2%, myeloproliferative disorders in 18.1%, chronic lymphocytic leukemia in 6.0%, cirrhosis in 6.0%, solid organ malignancies in 6.0%, chronic obstructive pulmonary disease in 3.0%, multiple myeloma in 3.0%, and Waldenström's macroglobulinemia in 3.0%. Similarly, the right renal atrophy cases have splenomegaly in 20.0%, thalassemia minors in 40.0%, and SCDs in 20.0%.

Conclusion: Renal atrophy is significantly higher on the left side. Aortic pressure induced flow disorders in the left renal vein, structural anomalies of the left renal vein including nutcracker syndrome and passage behind the aorta, and possibly the higher arterial pressure of the left kidney due to the shorter distance to the heart as an underlying etiology of endothelial damage and atherosclerosis may be some of the possible causes. Due to the stronger arterial walls protecting themselves from compression and high prevalences of splenomegaly and left varicocele in population, splenomegaly induced flow disorders of the left renal vein may be the most significant cause among all.

Key words: Left renal atrophy, splenomegaly, left renal vein, atherosclerosis.

Introduction

Atherosclerosis, but not venosclerosis, is an inflammatory process developing due to the higher arterial pressure that causes chronic endothelial damage in a lifelong period. It may be the major cause of aging and end-organ failures in human body (1,2). Probably, it is a systemic and irreversible process initiating at birth, and accelerated with many factors. The accelerating factors known for today are collected under the heading of metabolic syndrome. Some reversible components of the syndrome are overweight, hypertriglyceridemia, hyperbetalipoproteinemia, dyslipidemia, white coat hypertension, impaired fasting glucose, impaired glucose tolerance, and smoking for the development of terminal consequences such as obesity, diabetes mellitus, hypertension, coronary heart disease, chronic obstructive pulmonary disease (COPD), cirrhosis, chronic renal disease (CRD), peripheric artery disease, stroke, and other end-organ failures (3-8). On the other hand, nephrons are the basic functional units of the kidney located in the renal parenchyma, and renal atrophy is characterized by shrinkage of kidney due to loss of nephrons. Several kinds of primary renal diseases and acute or chronic pyelonephritis may cause renal atrophy. Renal atrophy may also be teminated by the obstruction of urinary tract due to an increased pressure on it. Obstructive uropathy causes a higher urinary pressure within the kidneys causing damage to the nephrons. Although the presence of several possible causes, the most common cause of renal atrophy may be the systemic atherosclerosis, and CRD due to the metabolic syndrome is common in elderlies. Sickle cell diseases (SCDs) is another accelerated systemic atherosclerotic process, and the higher prevalence of CRD in SCD patients may also indicate the underlying atherosclerotic background of CRD (9). We tried to understand whether or not there is a difference according to renal atrophy between the left and right sides in human body in the present study.

Material and methods

The study was performed in the Hematology Service of the Mustafa Kemal University between March 2007 and July 2013. All patients applying to the service were enrolled into the study. A check up procedure including serum iron, total iron binding capacity, serum ferritin, hepatic function tests, markers of hepatitis viruses A, B, and C and human immunodeficiency virus, and an abdominal ultrasonography was performed. Renal atrophies and cases with splenomegaly were detected, ultrasonographically. Thalassemias are diagnosed by serum iron, total iron binding capacity, serum ferritin, and the hemoglobin electrophoresis performed via high performance liquid chromatography (HPLC) method. SCDs are diagnosed by the hemoglobin electrophoresis performed via high performance liquid chromatography (HPLC) method. The criterion for diagnosis of COPD is post-bronchodilator forced expiratory volume in 1 second/forced vital capacity of less than 70% (10). Cirrhosis is diagnosed with hepatic function tests, ultrasonographic findings, ascites, and histologic procedure in case of requirement. Finally, the left and right renal atrophy cases were compared in between according to the general prevalences in the Hematology Service and associated disorders with them. Mann-Whitney U test, Independent-Samples t test, and comparison of proportions were used as the methods of statistical analyses.

Results

The study included 2.417 cases (1.248 females and 1.169 males). The mean ages of them were 47.3 ± 20.1 (8-105) versus 50.7 ± 20.4 (6-105) years in females and males, respectively (p<0.000). Interestingly, there were 33 cases (1.3%) with the left renal atrophy against only five cases (0.2%) with the right (p<0.001) among the study cases. The female ratios were 39.3% (13) and 40.0% (2) in the left and right renal atrophy cases, respectively (p>0.05). The left renal atrophy cases have splenomegaly in 51.5% (17), thalassemia minors in 30.3% (10), SCDs in 27.2% (9), myeloproliferative disorders including polycythemia vera, chronic myelocytic leukemia, and essential thrombocytosis in 18.1% (6), chronic lymphocytic leukemia in 6.0% (2), cirrhosis in 6.0% (2), solid organ malignancies in 6.0% (2), COPD in 3.0% (1), multiple myeloma in 3.0% (1), Waldenström's macroglobulinemia in 3.0% (1), and cyst hydatid in 3.0% (1) of them (Table 1). Similarly, the right renal atrophy cases have splenomegaly in 20.0% (1), thalassemia minors in 40.0% (2), and SCDs in 20.0% (1) of them (Table 2).

Table 1. Left renal atrophy cases with associated disorders

Variables	Prevalence
Splenomegaly	51.5% (17)
Thalassemia minors	30.3% (10)
Sickle cell diseases	27.2% (9)
Chronic myeloproliferative disorders	18.1% (6)
Chronic lymphocytic leukemia	6.0% (2)
Cirrhosis	6.0% (2)
Solid organ malignancies	6.0% (2)
Chronic obstructive pulmonary disease	3.0% (1)
Multiple myeloma	3.0% (1)
Waldenstrom's macroglobulinemia	3.0% (1)
Cyst hydatid	3.0% (1)

Table 2. Right renal atrophy cases with associated disorders

Variables	Prevalence
Splenomegaly	20.0% (1)
Thalassemia minors	40.0% (2)
Sickle cell diseases	20.0% (1)

Discussion

Thalassemias are chronic hemolytic anemias, and 1.6% of the population are heterozygous for alpha- or beta-thalassemias in the world (11). So thalassemias are probably the most common cause of splenomegaly in population. Interestingly, we detected the prevalences of thalassemia minors as 30.3% and 40.0% in the left and right renal atrophy cases, respectively, in the present study. They are autosomal recessively inherited disorders as in SCDs. It results from unbalanced hemoglobin synthesis caused by decreased production of at least one globin polypeptide chain (alpha, beta or delta). Alpha-thalassemias result from decreased alpha chain synthesis and beta-thalassemias from decreased beta chain synthesis. A mild to moderate anemia induced tissue hypoxia, bone marrow hyperactivity due to the chronic hemolytic proce-

ss, splenic hyperactivity and splenomegaly, cardiopulmonary hyperactivity, and growth retardation may develop in the thalassemia cases.

SCDs are accelerated systemic atherosclerotic processes initiating at birth (12,13), and by which we can observe final consequences of the systemic atherosclerosis nearly 30 or 40 years earlier in the life. SCDs are caused by homozygous inheritance of the hemoglobin S (Hb S). Hb S causes erythrocytes to change their normal elastic structures to hard bodies. Actually, the name should be 'Rigid Cell Induced Chronic Endothelial Dysfunction Syndrome' since we can not observe the sickle cells in the peripheric blood samples of cases with additional thalassemias, easily. So, the rigidity of erythrocytes is the main problem instead of their shapes or severity of anemia. The rigidity process is probably present in whole life but exaggerated with various stresses. The erythrocytes can take their normal elastic structures after normalization of the stresses, but after repeated attackes of rigidity, they become hard bodies, permanently. The rigid cells induced chronic endothelial damage causes tissue ischemia, infarction, and end-organ failures even in the absence of obvious vascular occlusions on the chronic background of edematous and damaged endothelium all over the body. Probably, there is not any organ in the body that can protect itself from damage of the rigid cells. The digital clubbing and recurrent leg ulcers may also indicate the chronic tissue hypoxia in such individuals. Due to the reversibility of digital clubbing and leg ulcers with the hydroxyurea treatment (14), the chronic endothelial damage is probably prominent at the microvascular level as in diabetic microangiopaties, and reversible with some extent. Although large arteries and arteriols are especially important for blood transport, capillaries are more important for tissue oxygenation. So passage of the rigid cells through the endothelial cells particularly cause damage on the capillaries. Reversibility of the process may probably be more in early years of the life but it gets an irreversible nature by time. Splenomegaly is also frequent in early years of the life due to the edematous and swollen endothelium in the spleen. But the ischemic process terminates with tissue fibrosis and shrinkage all over the body as in autosplenectomy. The case of right renal atrophy together with the SCDs may also be explained by the mechanism in the present study. Eventually, the mean survivals were 42 and 48 years for males and females for the SCDs in the literature (15). On the other hand, anemia probably is not the cause of the end-organ failures in the SCDs, since we can not observe any shortened survival in the thalassemia minors although the presence of a moderate anemia.

Varicocele is a dilatation of pampiniform venous plexus within scrotum. It occurs in 15-20% of all males and 40% of infertile males, since researchers documented a recurrent pattern of low sperm count, poor motility, and predominance of abnormal sperm forms in varicocele cases (16,17). Varicoceles are much more common in the left side (nearly 80% to 90%) due to several anatomic factors including angle at which the left testicular vein enters the left renal vein, lack of effective antireflux valves at the juncture of left testicular vein and left renal vein, the nutcracker syndrome, and some other left renal vein anomalies such as passage behind the aorta (18,19). The nutcracker syndrome results mostly from the compression of the left renal vein between the abdominal aorta and superior mesenteric artery, although some other variants exist (20). But according to our opinion, the higher prevalences of varicocele on the left side may mainly be a result of high prevalences of thalassemia minors and splenomegaly in population that may cause drainage problems in the left renal vein.

The accelerated atherosclerotic process can also affect the renal arteries, and may lead to poor perfusion of the kidneys leading to renal failure. The right renal artery is longer than the left because of the location of the aorta. Additionally, the right renal artery is lower than the left because of the position of the right kidney. So the left kidney possibly has a relatively higher arterial pressure due to the shorter distance to the heart as an underlying cause of endothelial damage and atherosclerosis. But according to our opinion, the accelerated atherosclerotic process alone can not explain the significantly higher prevalence of renal atrophy on the left side (1.3% versus 0.2%, $p<0.001$) in the present study. The left renal atrophy has also been reported in the literature (21). On the other hand, the high prevalences of associated thalassemias (30.3%) and splenomegaly (51.5%) with the left

renal atrophy cases may be important for the explanation, since spleen and left kidney are closely related organs which may also be observed with the development of varicose veins from the left renal vein at the splenic hilus in cirrhotics. Any pressure on the left kidney as in splenomegaly cases may cause torsion of the left renal vein, and prevents its drainage. We especially think about the drainage problems at the venous level due to the stronger arterial walls that can not be obstructed easily and the higher prevalence of varicocele in the left side in males (22, 23).

As a conclusion, the renal atrophy is significantly higher on the left side. Aortic pressure induced flow disorders in the left renal vein, structural anomalies of the left renal vein including nutcracker syndrome and passage behind the aorta, and possibly the higher arterial pressure of the left kidney due to the shorter distance to the heart as an underlying etiology of endothelial damage and atherosclerosis may be some of the possible causes. Due to the stronger arterial walls protecting themselves from compression and high prevalences of splenomegaly and left varicocele in population, splenomegaly induced flow disorders of the left renal vein may be the most significant cause among all.

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Thymectomy in central lymph node dissection for papillary thyroid cancer

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Abstract

Background: Central lymph node dissection (CND) has been proposed in the treatment of patients affected by papillary thyroid cancer(PTC) with clinically negative neck lymph nodes. The procedure allows pathologic staging of lymph nodes of the central compartment and treatment of the micrometastases. By comparing bilateral and unilateral thymectomy during total thyroidectomy with central lymph node dissection for postoperative complications in sonographically node-negative papillary thyroid carcinomas, we aimed to determine the optimal extent of prophylactic central lymph node dissection.

Methods: Patients were divided into two study groups: Group 1, total thyroidectomy plus unilateral thymectomy during the CND; Group 2, total thyroidectomy associated with bilateral thymectomy (both upper poles) during the CND. Primary endpoints of the study were evaluated by comparing the postoperative complications between the two groups.

Results: The only significant result found when comparing the two groups was the rate of transient hypocalcemia. (Group 1: 13.7%, Group 2: 52.4%, $p<0.01$). A total of five cases of papillary thymic metastases were found in this study. And final pathology confirmed that all cases of thymic metastases were lymph node micrometastases of PTC, only situated in the ipsilateral thymus upper pole.

Conclusions: Bilateral thymectomy during the CND did not provide a better carcinologic resection, as no contralateral thymic metastases were found. The unilateral thymectomy with total thyroidectomy during the CND may represent an effective strategy for reducing the rate of postoperative hypocalcemia.

Key words: Thymectomy, papillary thyroid cancer, lymph node dissection.

Introduction

Central lymph node dissection(CND) is a common adjunct to thyroidectomy in the treatment of papillary thyroid cancer(PTC)[1]. However, Postoperative hypocalcemia occurred commonly in this central dissection, which is related to the resection or devascularization of the inferior parathyroids together with thymectomy[2]. Because of the same embryologic origin of the thymus and the inferior parathyroids, they are in close proximity to each other and share blood supply[3, 4]. Both the thymus upper poles and the inferior parathyroid glands are located within the paratracheal area of the central compartment of the neck. This explains the increased postoperative hypocalcemia after a central lymph node dissection. Recent reports indicate thyroidectomy plus lymph node dissection resulted in 3% to 6% of PTC patients suffering postoperative hypocalcemia [5, 6]. In order to minimize this complication, some surgeons perform unilateral thymectomy and preserve the thymus contralateral to the cancer[7]. However, few reports to date have closely analyzed postoperative hypocalcemia after thyroidectomy plus node dissection.

By comparing bilateral and unilateral thymectomy during total thyroidectomy with central compartment lymph node dissection for postoperative complications in sonographically node-negative papillary thyroid carcinomas, we aimed to determine the optimal extent of prophylactic central compartment lymph node dissection.

Methods

From 2009 to 2012, a total of 155 consecutive patients, diagnosed as sonographically node-negative thyroid papillary carcinomas, were included in this study. All subjects underwent total thyroidectomy in conjunction with one of two types of

central compartment lymph node dissection (lymph node level VI). Patients were excluded from the study if the patients who had undergone (1) previous thyroid or parathyroid surgery; (2)a unilateral lobectomy, subtotal, or completion thyroidectomy; (3) previous neck irradiation; and (4)concomitant surgery for hyperparathyroidism. The diagnosis of papillary thyroid carcinomas was reconfirmed by the surgical pathology in all patients.

The CND was carried out in a conventional manner and was well described and illustrated by Grodski et al[8]. Including all perithyroidal and paratracheal soft tissue and lymph nodes with borders extending superiorly to the hyoid bone, inferiorly to the innominate artery, and laterally to the common carotid arteries.

Patients were divided into two groups according to CND: One in whom patients had a total thyroidectomy plus unilateral thymectomy during the CND, leaving in situ the contralateral thymus upper pole. While the other group had a total thyroidectomy associated with bilateral thymectomy (both upper poles) during the CND. And the characteristics of the patients are presented in Table 1. Both groups' characteristics, including age, gender, MACIS score and tumor size, were comparable.

All patients were clinically evaluated for signs and symptoms of hypocalcemia. Transient hypocalcemia was defined as a symptomatic patient with an serum calcium level lower than 8.0 mg/dL at any time during the hospital stay[9]. While permanent hypocalcemia was defined as persistent symptomatic or biochemical (Ca level below 8.0 mg/dL) hypocalcemia at more than 6 months after surgery [9].

SPSS 12.0 for windows (SPSS Inc., Chicago, IL) was used for statistical analysis. Student *t* test was used when comparing continuous variables between groups; Statistical analysis of the differences in clinical variables between groups was conducted by the Chi-square test. A two-tailed *P* less than 0.05 was considered to be significant.

Results

As showed in Table 2, 73 patients had unilateral(ipsilateral) thymectomy(Group 1) and 82 patients had bilateral thymectomy(Group 2). There were no significant differences in the number of inferior parathyroid autotransplantation in the sternocleidomastoid muscle between the two groups(*P*=0.657). Transient hypocalcemia was documented in 10 patients (13.7%) in Group 1, in

Table 1. Patient demographics and clinical characteristics

Variable	Group1 (n=73)	Group2 (n=82)	P*
Gender(M/F)	11/62	17/65	0.360
Age			
Mean±SD	48.1±10.7	48.7±10.4	0.770
Groups(<45/>45)	22/51	45/37	0.059
MACIS score			
Mean±SD	4.7±0.9	4.9±1.0	0.360
High vs low risk (<6 /> 6)	7/73	16/82	0.083
Tumor size(cm)			
Mean±SD	2.76±1.23	2.56±1.20	0.206

Group 1: Unilateral thymectomy group; Group 2: Bilateral thymectomy group.
**test except for age, MACIS score, and tumor size(t test). MACIS indicates metastasis, age, completeness, invasiveness, size.*

Table 2. Comparative results between the two groups

Variable	Group 1 (n=73)	Group 2 (n=82)	P*
Parathyroid autotransplantation	5.1±1.5	5.2±1.3	0.657
Hypocalcemia			
Transient	10(13.7%)	43(52.4%)	<0.01
Permanent	0(0%)	3(3.6%)	0.099
Thymus metastases	2(2.7%)	3(3.6%)	0.747

Group 1: Unilateral thymectomy group; Group 2: Bilateral thymectomy group.
**test except for Parathyroid autotransplantation(t test).*

43 patients(52.4%) in Group 2. Statistical analysis showed significant difference(P<0.01) in the rate of transient hypocalcemia between two groups. However, there were no significant differences in the rate of permanent hypocalcemia between two groups(P=0.099). Both Group1 and Group2 were found to have cases of thymic metastases(2.7%, 3.6% respectively), this difference did not reach statistical significance(P=0.747). Interestingly, final pathology examination confirmed that the five cases of thymic metastases were lymph node micrometastases of PTC, only situated in the ipsilateral thymus upper pole. No contralateral thymic metastases were found in either group.

Discussion

PTC is the most common thyroid malignancy and carries an excellent prognosis. While up to 20–90% patients with PTC may have lymph node metastasis detected during the initial surgery[10]. Lymph node metastases generally occur in a stepwise fashion and the central neck is the most common site of metastatic PTC[8, 11, 12]. In this study, pathologically confirmed central neck lymph nodes metastasis from PTC occurs commonly. In contrast to the preoperative assessment of the central compartment lymph nodes with ultrasound. Our results are consistent with previously reported study[13]. Which indicating preoperative ultrasonography is an unreliable technique for detecting the central lymph node metastasis, this might be because the air-filled trachea as well as the fatty tissue containing the lymphatic tissue disturbed ultrasonography detection of lymph nodes that were small. Furthermore, suggesting prophylactic central neck dissection should be carried out for the management of cN0 PTC.

The thymus is an important organ involved in cellmediated immunological function. The parenchyma of the thymus has a blood-thymus barrier[14], which prevents the thymus from making direct contact with antigens or cancerous cells, thereby it has been considered almost impossible that a tumor could metastasize to the thymus. However, in the present study, five cases of thymic metastasis from PTC were identified by final pathology, within the thymus upper poles. Our results are consistent with previously reported studies[15-17], and might be explained because blood-thymus barrier is not as robust in the medulla of the organ as it is in the cortex, when the structure of the thymus is precisely analyzed. The septum of the thymus is comprised of interlobular connective tissue with blood vessels, lymphoducts and nerves, which theoretically does not exclude the possibility of metastasis[14]. And we presumed that because of the lymphatic metastasis behavior of the PTC[18], the mechanism of metastasis by the thyroid cancer to thymic may have been via a lymphatic pathway. However, whether the location of the tumor suggested metastasis via a lymphatic pathway or local venous pathway, needs further study.

When CND was performed, the fibrofatty tissue in the midline is incised to expose the trachea down to the level of the brachiocephalic vessels inferiorly, and the medial border of the carotid artery is dissected down to the prevertebral fascia. The superior limit of dissection is at the level of the cricoid cartilage, and the thymus is transected at the level of the brachiocephalic vessels and the specimen is removed[7, 19] Anatomically, both the inferior parathyroid gland and the thymus upper poles are located in the central compartment of neck. Embryologically, the thymus gland originates from the third pair of branchial pouches high in the neck during early foetal life and start their descent toward the mediastinum. And the inferior parathyroid gland has the common embryologic origin, which is responsible for the close relationship and the shared blood supply between both glands[20]. In our study, the unilateral group had fewer transient hypocalcemia cases than the bilateral group. Suggesting that the increased rate of postoperative hypocalcemia is more related to bilateral thymectomy, with removal or devascularization of the inferior parathyroid glands during a central neck dissection. Furthermore, all of the thymic metastases were found in the side of the tumor, no contralateral thymic metastases were found in either group. Our results are consistent with previously reported studies[17, 21]. Indicating that the unilateral thymectomy provides enough carcinologic resection. It is necessary to differentiate the thymus upper pole in both side and preserve the thymus contralateral to the cancer with the goal of keeping a better parathyroid function.

The main limitation of the study is represented by the fact that, this is a retrospective observational study. Thus, a large randomized trial with long-term follow-up is needed, however, the low prevalence of PTC combined with its relatively indolent nature has meant that ideal treatment research protocols are difficult to undertake.

In summary, the unilateral thymectomy with total thyroidectomy could be an alternative surgical option for clinically node-negative papillary thyroid carcinomas, because it results in a reduced transient postoperative hypocalcemia and similar carcinologic resection. Further study with a larger number of patients and long-term follow-up is needed.

Authors' contributions

XHY, XYQ and XHZH analyzed and collected the patient data. DPH was a major contributor in writing the manuscript. All authors read and approved the final manuscript."

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The use of a harmonic scalpel in surgical management of hyperthyroidism

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Abstract

Background: The aim of our study was to compare total thyroidectomy due to hyperthyroidism, performed with the harmonic scalpel (HS) and with the classic clamp-and-tie technique (SL) in terms of safety, surgical time and postoperative complications**.**

Methods: Data from 97 patients with hyperthyroidism who underwent total thyroidectomies have been recorded from 2008 to 2011.

Results: A statistically significant reduction of surgical time were found for groups HS vs.SL (103.3 vs. 78.3 minutes) and for reduction of intraoperative blood loss (144mL vs. 88 mL). There was a significant difference between the two groups in the severity of postoperative pain, the number of analgesia intake in the first 48 h as well as intervals of pain-free return to normal activity and return to work.

Conclusion: HS can reduce operative time and minimize intraoperative blood loss with a significant modification of postoperative outcome.

Key words: Harmonic scalpel, Total thyroidectomy, hyperthyroidism.

Introduction

Thyroid surgery consists of the meticulous devascularization of the thyroid gland, the richest with blood supplies among the organs. Hemostasis, thus, is essential for the control and division of various vessels before the gland excision (1). During thyroidectomy, hemostasis was usually performed using clamp-and-tie maneuvers for ligation of this and many smaller thyroid vessels. Other applicable methods were clips and electrocautery. Newly developed hemostatic device is the harmonic scalpel. It is designed as an alternative to conventional vessel sealing techniques. The harmonic scalpel offers adequate hemostasis with minimal thermal spread, minimal adjacent tissue destruction, and no foreign bodies (i.e., ligations or clips) left behind (2,3).

Thyroidectomy requires numerous clamp and tie maneuvers for the small thyroid vessels. Therefore, the use of the harmonic scalpel in thyroid surgery may reduce surgical time, cost, and morbidity.

If surgery is considered for definitive management of hyperthyroidism, total thyroidectomy is the surgical technique of choice.

The aim of our study was to compare total thyroidectomy due to hyperthyroidism, performed with the harmonic scalpel and with the classic clamp-and-tie technique in terms of safety, surgical time and postoperative complications.

Materials and Methods

Data from all patients with hyperthyroidism who underwent total thyroidectomies performed by classic suture ligation technique or with the harmonic scalpel (Ultracision, CS-14C; Ethicon) in the Department of Endocrine Surgery have been recorded from 2008 to 2011.

In the period from January 2008 to May 2009 hemostasis in these surgeries was performed with the conventional knot-and-tie technique (group I). In all patients who had surgery between Jun 2009 and August 2011, hemostasis was achieved with the harmonic scalpel, (group II). To reduce potential bias due to interoperator variability, all operations were performed by the same surgeon team who had experience in thyroid surgery.

The Conventional Knot-and-tie Technique: formal thyroid vessels (inferior middle and superior) were clamped and tied using 3/0 silk sutures, and in all other vessels absorbable 2/0 or 3/0 sutures were used. The harmonic scalpel was used for the ligation of all thyroid vessels up to 3 mm

in diameter apart from the superior thyroid artery, which was ligated with the conventional knot-and-tie technique (3/0 silk sutures). The generator of the ultrasonic device was adjusted to a minimum power level of 3 and a maximum power level of 5.

We excluded patients undergoing re-operation. Patients were treated with an initial dose of propranolol (40-60 mg/day) plus propylthiouracil (300-400 mg/day) or methimazole (10-30 mg/day), which were reduced gradually to maintain euthyroidism as serum thyroid hormone concentrations declined.

Preoperative medication with Lugol's solution (iodine-iodide solution) 2% (2 g/100 mL of iodine and 4 g/100 mL of iodide) was combined with the preexisting antithyroid therapy according to a schedule of oral administration at increasing dosages starting 7 days before operation and reaching a total dose of 75 mg of iodine and 150 mg of iodide (75 drops corresponding to 3.75 mL). On the first day, 5 drops were administered, on the second day 7 drops, and so on, reaching 15 drops on the sixth and seventh days.

It is a common practice in our department to place a suction drain in all patients that is removed on the second postoperative day. Serum calcium level was determined 12 and 24 hours postoperatively. Patients with dyspnea, dysphagia, or voice alterations such as hoarseness, easy voice fatigue during phonation, and difficulty with high pitch and singing voice were evaluated with laryngoscopy. Uncomplicated cases were discharged on the second postoperative day when calcium levels were within the normal range (8.5– 10.5 mg/dL). All patients were followed up at 1, 2, and 4 weeks after surgery.

Operative time, which was measured from the moment of the incision to the last skin stitch, time was calculated by surgery. The medical records of the patients enrolled were reviewed and compared regarding age, sex, diagnosis, estimated intraoperative blood loss, postoperative complications and hospital stay.

Calcium gluconate infusion (10 mL in 100 mL of 0.9% saline solution, IV, every 12 hours) was used if patients presented symptomatic hypocalcemia (defined as generalized paresthesia, carpopedal spasms, or respiratory distress), and oral calcium was used on the first postoperative day if patients were asymptomatic but presented total serum calcium <7.5 mg/dL

Postoperative pain was assisted for the first 2 postoperative days using the visual analogue scale (VAS) as a part of our routine protocol. Every patient was learned how to mark the level of his pain daily on a chart given to him and to present this chart at each follow-up visit. The chart was graded from 0 to 10, marked at one end as no pain and at the other end as worst pain imaginable. Patients with scores of 3 or less were defined as having mild pain, those with score of 7 or more were considered to have severe pain and those from 3.1 to 6.9 moderate pain. Also, the amounts of narcotics needed to relieve pain were assessed. Outcome evaluation was measured by the duration of return to activity, pain-free to return to activity, return to work and pain-free to return to work.

Data were analyzed with the use of the SPSS 10.0 statistical package (Microsoft Corp) Statistical analysis was performed using analysis of variance and the Kruskal-Wallis test for numeric variables, and the chi-square test and the Fisher exact test for categoric variables. Data were expressed as means (SD) unless otherwise specified. The level of statistical significance was set at a *P* value of less than .05.

Results

During the study period, 97 consecutive patients underwent total thyroidectomy by the same endocrine surgical team. Hemostasis was achieved using either the traditional suture ligation over clamp procedure (group SL, n=46) or Ultracision Harmonic Scalpel (group HS, n=51). The 2 groups were comparable in terms of age, sex, and pre and postoperative serum calcium levels, surgical time, type of diagnosis, intraoperative blood loss and hospital stay (Table 1).

Total thyroidectomy was completed in all cases. A statistically significant reduction of surgical time was found for groups HS compared with group SL from 103.3 minutes to about 78.3 minutes Also a statistically significant reduction of intraoperative blood loss was found for groups HS compared with group SL from 144 mL to about 88 mL (Table 1).

In contrast, no difference was found in age and sex between groups SL and HS. There were no differences in the 2 groups regarding thyroid

gland weight or diagnosis, or pre and postoperative calcium levels. (Table 1).

In the SL group, three patients suffered transient hypocalcaemia, one patient developed wound seroma, and three patients developed transient hoarseness, and all were resolved within 3 months. In the U group, three patients developed transient hypocalcemia, two patients suffered transient hoarseness of voice and one patient developed wound seroma. There was no permanent complication in both groups.

Significant postoperative bleeding that lead to re-operation occurred in 1 case in the conventional knot-and-tie technique group. The bleeding point was located in the superior thyroid artery. Patient had an uneventful recovery.

There was a significant difference between the two groups in the severity of postoperative pain as well as the number of analgesia intake in the first 48 h. Moreover, we have found that there was a significant difference between both groups regarding the intervals of pain-free return to normal activity and return to work (Table 2).

Discussion

There have been few developments in the technical aspects of thyroid surgery since the surgical approach described by Kocher greater than a century ago (4) The Harmonic Scalpel was introduced into the surgeon's armamentarium almost two deca-

Table 1. Clinical and demographic characteristics of the patients

Variable	Group SL (n= 46)	Group HS (n=51)	*P* value
Age,y*	48.3(11.2)	50.5(13.3)	ANOVA,NS
Sex, (n%) Male Female	 15(32.6) 31(67.4)	 14(27.45) 36(72.55)	Chi-square NS
Diagnosis, n (%) Toxic nodular goiter Grave's disease	 34(73.91) 12(26.09)	 40(80.0) 10(20.0)	Chi-square, NS
Specimen weight, (g)*	85.5 (23.6)	79.3(30.1)	Kruskal-Wallis, NS
Preoperative calcium level, (mg/dL)*	9.3(0.4)	9.4(0.5)	Kruskal-Wallis,NS
Surgical time, min*	103.3 (14.5)	78.3 (11.2)	Kruskal-Wallis <0.001
Postoperative calcium level, (mg/dL)*	8.9(0.4)	9.1(0.5)	Kruskal-Wallis, NS
Intraoperative blood loss, (mL)*	144(16)	88(11)	Kruskal-Wallis, <0.001
Hospital stay, (days)*	3.1(0.5)	2.8(0.5)	Kruskal-Wallis, NS

NS= not statistically significant.
** Values are expressed as means (SD).*

Table 2. Comparison of postoperative complications, analgesic intake and visual analogue scale

Variable	Group SL (n=46)	Group HS (n= 51)	*P* value
Transient complications n (%)	8(17.39)	6(11.76)	Chi-square,NS
Transient hypocalcaemia n (%)	3(6.52)	3(5.88)	
Wound seroma n (%)	1(2.17)	1(1.96)	
Hoarseness n (%)	3(6.52)	2(3.92)	
Bleeding n (%)	1(2.17)	0(0)	
Permanent complications n (%)	0	0	
Analgesic intake (n)	2.1(0.5)	1.3(0.3)	Chi-square,0.05
Visual analogue scale	4.9(1.3)	3.1(1.3)	Chi-square,0.09
Pain-free to return to activity (days) *	13.5(8.7)	8.7(4.2)	Kruskal-Wallis <0.001
Pain-free to return to work (days) *	23.8(6.4)	17.4(2.2)	Kruskal-Wallis <0.001

NS= not statistically significant.
** Values are expressed as means (SD).*

des ago. Using mechanical vibrations at 55.5 kHz, this device is able to cut and coagulate tissue simultaneously. The proposed advantages of using this device over traditional electrocautery include less lateral thermal tissue injury, a lack of neuromuscular stimulation, and the avoidance of electrical energy transmission either to or through the patient (5).

Food and Drug Administration approved the Ultracision harmonic scalpel for ligation of vessels up to 3 mm in diameter. Thermal damage is limited to 0 to 2 mm beyond the tissue grasped within the forceps of the device (6-8). Although the use of the harmonic scalpel for the superior thyroid artery is under concern, there are several reports that prove the safety and efficiency of the device for this vessel (9-10). We legated the superior thyroid artery with the conventional knot-and-tie technique (3/0 silk sutures) in our group of patients . Hemostasis in thyroid surgery avoids injuries to the parathyroid glands and the superior and inferior laryngeal nerves, as well as life-threatening postoperative hematomas.

There are many reports, carried out at European centers, that evaluate the utility of the HS for thyroid surgery. The results regarding reduced operative times and its utilization were similar, but those regarding other postoperative outcomes such as transient postoperative hypocalcemia and recurrent laryngeal nerve dysfunction (RLND) differed. These complications are relatively uncommon and the number of cases reported in individual studies is limited. Data consolidation may allow for elucidation of significant associations between HS utilization and postoperative complications.

The purpose of this study was to determine whether conventional hemostasis (CH) or the HS results in shorter operative times for thyroidectomy and to evaluate the incidence of postoperative complications with each approach.

There are some reports of the increased risk of postoperative hypocalcaemia with conventional hemostasis techniques. Only one report had a cohort large enough and statistically significant association (11, 13). Though the mechanism is not fully understood, transient hypocalcaemia observed after total thyroidectomy is believed to be associated with traumatization of the parathyroid glands, which are anatomically closely connected to the thyroid gland and share its blood supplies. We believe that the use of the HS facilitates parathyroid gland dissection away from the parathyroid gland capsule.

In our study transient postoperative hypocalcaemia with HS was found in 3(6.52) patients compared to SL techniques 3(5.88) patients and there was also no statistically significant difference in the risk of transient postoperative RLND between the two groups (SL 2.17% vs. HS 1.96%). The complication of RLND after thyroidectomy is also an extremely uncommon occurrence. Included studies had conflicting results in terms of the risks of RLND with HS utilization compared to CH, and all reported either very few or no cases of this complication. Given that HS has been shown to cause less collateral thermal injury than conventional electrocautery, we would expect to see less RLND in the HS group. Unfortunately, the numbers in this analysis are too small to generate any meaningful conclusions. No case of permanent RLND occurred in both groups.

According to the length of surgical procedure all studies found that thyroidectomy was faster with the HS, they were quite heterogeneous in terms of the baseline length of time required to carry out a conventional thyroidectomy (range from 46.7 to 168.8 minutes). This observed difference in time required to carry out the same operation is quite striking. The heterogeneity may have been due to the size of the gland that was being resected, which was not clearly defined in all studies. In addition, all of the thyoidectomies in the Hallgrimsson study (12), which reported the longest mean operative time for conventional thyroidectomy, were carried out for Graves' thyrotoxicosis, wherein the vascularization of the thyroid gland can be very extensive. In contrast, the majority of thyroidectomies in the study reporting the fastest mean operative time excluded patients with Graves' disease or extensive goiters (13,14,15).Our results shoed that we have longer operative time in both groups but total thyroidectomy performed with HS were faster HS 103.3min vs. SL 78.3 min).

In the recent time Kowalski LP et al. (16) designed an open, phase IV, multicenter, randomized controlled trial that compared the use of an ultrasonic scalpel with a conventional technique in patients with total thyroidectomy. The outcomes were surgical complication rate, operative time, drainage volume, postoperative pain, and costs. In all, 261 patients

were included in 11 centers. There was a mean difference of 17% of operative time in favor of the ultrasonic scalpel group. There were no differences in postoperative complications. There was a difference in costs of 14% in favor of the ultrasonic scalpel group, but it was not statistically significant. The use of an ultrasonic scalpel was as safe as that of the conventional technique and had the advantage of a shorter operative time and lower postoperative drainage.

Efficacy of harmonic scalper was also proved postoperatively, since no wound hematoma was noticed in HS group of patients. The incidence of postoperative seroma was not different between the two groups.

Moreover, a significant difference between the two groups regarding the severity of postoperative pain and the amount of narcotics needed was proved. This may be due to minimal tissue dissection when using the Ultracision harmonic scalpel and the fact that Ultracision harmonic scalpel has a reduced energy spread profile when compared with unipolar cautery.

In conclusion, the use of the Ultracision harmonic scalpel in management of hyperthyroidism is equally as safe and effective as the conventional knot-tying technique. Moreover, the Ultracision harmonic scalpel can reduce operative time and minimize intraoperative blood loss with a significant modification of postoperative outcome regarding the postoperative pain severity, and earlier return to normal activity and work.

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Epidemic characteristics and associated climatic factors of the pandemic 2009 influenza A (H1N1) in Heilongjiang, China

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Abstract

Background: The pandemic influenza A(H1N1) virus emerged and had spread worldwide during 2009. It's yet not reported whether climatic factors could affect dissemination of H1N1.

Methods: We retrospectively analyzed all the 1923 cases of confirmed H1N1 in 2009，Heilongjiang ,China and the association between weekly climatic factors and H1N1 onset was evaluated by Pearson correlation.

Results: The average age of confirmed cases was 19.92±12.01 years old, and hospitalized cases was 27.1±17.2 years old. Analyzed according to the occupations, the students are the most susceptible (67.4%). Classified by region, the provincial capital Harbin constituted 54.8% incidence, while the edge region Daxinganling had the lowest onset (0.6%). Both Onset no. and admission no. of H1N1 presented 2 outbreak peaks, and admission peaks were just 1 week behind the onset ones. From all data of 13 regions, it could be found that the climatic factors correlative to H1N1 were temperature, airpressure and humidity. From data of Harbin region, temperature and airpressure of identical week showed significant correlation with onset no. of next week

Conclusion: Our summary of these H1N1 data is of importance for the prevention and controlling of H1N1, and the climatic factors might influence the outbreak and spreading of H1N1.

Key words: Influenza A (H1N1), Epidemic characteristic, Climatic factor.

Introduction

The influenza A (H1N1) virus was firstly confirmed diagnosed in the United States and Mexico in April 2009, and has spread worldwide rapidly[1,2]. As of 31 December 2009, more than 208 countries and overseas territories or communities have been involved, including at least 12220 deaths[3]. The first H1N1 case of China was confirmed in Beijing on 10 May, 2009. As of 28 February 2010 , More than 31 provinces and areas in China were affected, bringing out over 127,000 laboratory confirmed cases, and 793 deaths[4]. H1N1 differs from seasonal flu in their wide spreading, rapid progression, population susceptibility and clinical features, thus WHO raised the pandemic level from 5 to 6, the highest level[5]. Multiple factors could influence the rapid spreading of H1N1, including virulence of virus, population susceptibility and aggregation, public sanitation, preventive and control measures, vaccination and so on.By H1N1 monitoring we observed that, in autumn and winter seasons, the H1N1 incidence descended but admission severe cases raised. It's yet not reported whether climatic factors could affect H1N1 onset, and what was the key factor to affect activity and dissemination of H1N1.

As a northeast province of China, Heilongjiang is an alpine-cold region, which is advantageous to investigate the effects of climatic factors on H1N1. Harbin city, the provincial capital of Heilongjiang, reported the first imported case of H1N1 in 11 July 2009. Till September, H1N1 outbreak started spread around the whole province. In this paper we retrospectively analyzed all the 1923 cases of confirmed H1N1 from 11 July 2009(when

the first imported case was reported) to 27 December 2009, including 342 admission cases. The epidemic and spreading characteristics of H1N1 were summarized and described, the correlation between H1N1 onset no. and admission no. and data of climatic factors in 13 cities and regions of Heilongjiang province weekly was analyzed, so as to investigate the climatic conditions which might influence the outbreak and prevalence of H1N1 in Heilongjiang province.

Methods

Data collection and detection of H1N1

Data of confirmed H1N1 patients of Heilongjiang in 2009 were collected from Novel influenza A/H1N1 Surveillance information system and China Information System for Diseases control and prevention. This national network was organized to monitor infection with pandemic influenza A/H1N1 virus in China by Ministry of health of the people's republic of China. A national guideline, adapted from guidelines provided by the US，Centers for Disease Control and prevention, was published on May 9 and used to direct the surveillance, diagnosis, and treatment of the influenza A/H1N1[6]. The Center of Diseases Control (CDC) of Heilongjiang province supervises 13 city-level CDCs. Throat swab specimen of each suspected case in medical institutions was sent to local CDC, and the H1N1 virus was determined with a Real-Time RT-PCR kit (Beijing Kinghawk Pharmaceutical CO., LTD., Peking, China); meanwhile, the onset and admission information of confirmed cases were collected. We defined H1N1 onset as confirmed case detected by RT-PCR after initial influenza symptoms. The information of H1N1 was reported by medical institutions to each local CDC daily. When a H1N1 case was confirmed, the information would be reported to provincial CDC and registered to Novel influenza A/H1N1 Surveillance information system and China Information System for Diseases control and prevention every Sunday. These medical institutions comprised 64 hospitals above second-class and sentinel hospitals, keeping patients from 13 cities and 113 affiliated districts or countries under surveillance.The information of confirmed cases collected includes age, gender, occupation, onset time and site, confirmed time, admission or not, and so on. For the admission confirmed cases, their information also consists of admission time, admission site, discharge time, and death information. These data were classified according to first onset site of each case, and the weekly H1N1 onset no. and admission cases was counted.

The data of climatic factors were provided by Meteorological Information Center of Heilongjiang province. The daily climatic data of 13 regions from 31 August to 27 December were collected, including Maximum temperature, Minimum temperature, Temperature difference, Maximum air pressure, Minimum air pressure, Average air pressure, Fastest wind speed, Slowest wind speed, Average wind speed, Average humidity, Maximum humidity. The average value of each factor in each week was calculated and brought into correlation analysis with corresponding H1N1 onset no. and admission no. of each week. Data of density of population in 13 cities and regions were collected from annual report of Heilongjiang provincial statistic bureau.

Statistic analysis

Descriptive statistics included frequency analysis (percentages) for categorical variables and means and standard deviations or medians and interquartile ranges (IQRs) for continuous variables. Onset No. and admission No. of influenza A(H1N1) are enumeration data，while climate factors are measurement data. We performed Pearson correlation analysis to identify density of population or climate factors independently associated with H1N1 onset or in-hospital no. A two-tailed P value of less than 0.05 was considered to indicate statistical significance, except in the multivariate model, where a P value of less than 0.01 was considered to indicate statistical significance. The statistical analyses were conducted using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

1. Epidemic features of H1N1 onset in Heilongjiang province, China

A total of 1923 cases of H1N1 were reported from the first imported H1N1 case in 11 July 2009 to 27 December 2009.The detailed epidemic characteristics were listed in table 1: more male were infected, 97.5% of them are Han Chinese people, the others

are of ethnic minorities such as Manchu, Mongolians, Koreans; the average age is 19.92±12.01 years old, the top 3 susceptible age groups are 10-14 years old (22.4%), 15-19 years old (20.0%) and 20-24 years old (18.8%), the lowest susceptibility belongs to the age group of 60-64 years old(0.2%). Analyzed according to the occupations, the students(from primary to college) are the most susceptible (67.4%), then the house service workers (4.8%) and office workers (3.5%), the lowest susceptibility belongs to the Public attendants (0.1%).The infants took up 5.3% of all the cases, but the medical workers accounted for only 1%. Classified by different regions, the provincial capital Harbin area constituted 54.8% of the infected cases, followed by Qiqihar and Mudanjiang region (9.7% and 7.8%), the edge city Daxinganling had the lowest onset (0.6%).The summary of 2009 H1N1 onset distribution in Heilongjiang province was shown in figure 1.The capital Harbin city has the highest onset, the farther to the capital, the lower onset. After analysis of all the cases, the interval from initial influenza symptoms to confirmed diagnosis is 4.1±3.days, the IQR is 2-5days. The information of all the admitted cases was listed in table 2. In all 342 hospitalized cases, the male took up 57.9% with an average age of onset 27.1±17.2 years old. The hospitalized patients were mainly in Harbin, Mudanjiang and Daqing cities, comprising 45.3%, 19.9% and 7.6% of all the hospitalized patients respectively. The average interval from initial influenza symptoms to confirmed diagnosis was 5.4±4.9 days, the IQR is 2-8 days; the interval from initial symptoms to admission was 3.0±5.0 days, the IQR is 0-5 days. There was no significant correlation between density of population and H1N1 onset no. (R=0.530, P=0.063), or H1N1 admission no. (R=0.471, P=0.104).

Table 1. Epidemic characteristics of confirmed H1N1 patients in 2009, Heilongjiang, China

Characteristic	Value
Comfirmed	1923
Male sex-no.(%)	1164(60.53)
Race-no.(%)	
Han	1874(97.5)
Minor or other	49(2.5)
Age-yr	
Mean±SD	19.92±12.01
Range	1-87
Age group-no.(%)	
0-4yr	73(3.8)
5-9yr	294(15.3)
10-14yr	430(22.4)
15-19yr	385(20.0)
20-24yr	361(18.8)
25-29yr	99(5.1)
30-34yr	56(2.9)
35-39yr	67(3.5)
40-44yr	38(2.0)
45-49yr	39(2.0)
50-54yr	35(1.8)
55-59yr	25(1.3)
60-64yr	4(0.2)
65--yr	17(0.9)
Ocupation-no.(%)	
Student	1296(67.4)
Home economics	92(4.8)
Staff	68(3.5)
Worker	60(3.1)
Farmer	50(2.6)
Teacher	22(1.1)
Retiree	21(1.1)
Food	1(0.1)
Medical staff	19(1.0)
Commerce	18(0.9)
Public attendant	1(0.1)
Child	101(5.3)
Other	57(3.0)
Non-information	117(6.1)
Distribution-no.(%)	
Harbin	1053(54.8)
Qiqihar	187(9.7)
Mudanjiang	150(7.8)
Daqing	113(5.9)
Heihe	75(3.9)
Shuihua	71(3.7)
Jiamusi	70(3.6)
Yichun	69(3.6)
Hegang	53(2.8)
Shuangyashan	27(1.4)
Jixi	23(1.2)
Qitaihe	20(1.0)
Daxinganling	11(0.6)
Time from first symptoms to Diagnosis-days(Mean±SD)	4.1±3.5
Median	4
IQR	2-5

IQR denotes interquartile range

Figure 1. Distribution of 1923 confirmed cases with influenza A(H1N1) in 2009, Heilongjiang, China

Table 2. Epidemic characteristics of confirmed H1N1 in the 342 hospitalized patients

Hospitalized	342
Male sex-no.(%)	198(57.9)
Age-yr	
Mean±SD	27.1±17.2
Range	1-86
Distribution-no.(%)	
Harbin	155(45.3)
Qiqihar	17(4.97)
Mudanjiang	68(19.9)
Daqing	26(7.60)
Heihe	7(2.04)
Shuihua	10(2.92)
Jiamusi	14(4.1)
Yichun	7(2.04)
Hegang	14(4.1)
Shuangyashan	7(2.04)
Jixi	13(3.80)
Qitaihe	1(0.3)
Daxinganling	3(0.9)
Time from first symptoms to Diagnosis-days (Mean±SD)	5.4±4.9
Median	4
IQR	2-8
Time from first symptoms to Admission -days(Mean±SD)	3.0±5.0
Median	3
IQR	0-5

IQR denotes interquartile range

2. The association between H1N1 onset and climatic factors in Heilongjiang Province, China

The weekly H1N1 onsets of 2009 in Heilongjiang province were summarized in figure 2. 2 outbreak peaks could be found in the late September (9.14-9.20 and 9.21-9.27) and late October (10.12-10.18 and 10.19-10.25), and the previous peak was obviously higher than the latter. It's shown in figure 3, there were also 2 peaks in hospitalized cases (9.28-10.4 and 11.9-11.15), which were just 1 week behind the onset peaks. Our data also showed that admission peak value in November was higher and more persistent than the first one.

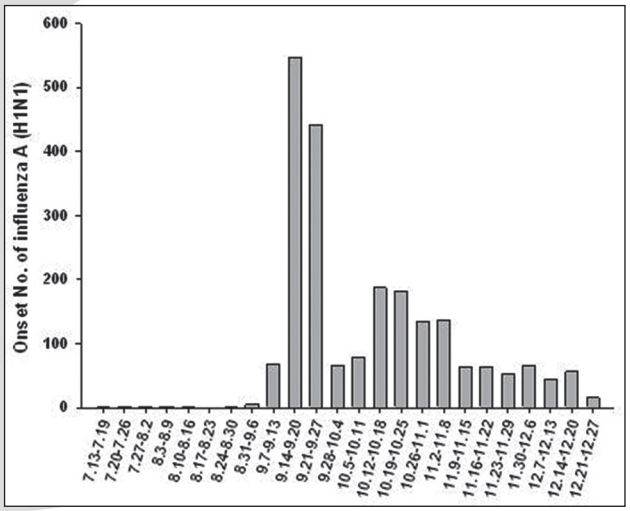

Figure 2. Onset no. of influenza A(H1N1) every week in Heilongjing during the study period

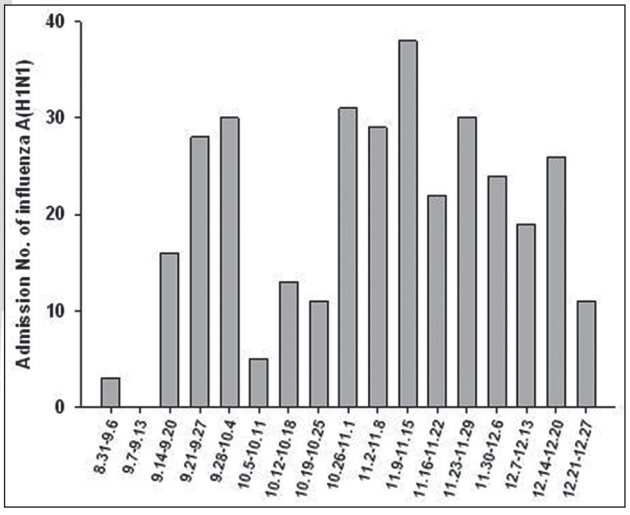

Figure 3. Admission no. of influenza A(H1N1) every week in Heilongjing during the study period

To investigate the association between climatic factors and H1N1 onset, we analyzed the weekly onsets of different cities and corresponding climatic

factors of Heilongjiang province in 2009. Table 3 revealed that the most significant climatic factors were maximum temperature (R=0.254, P=0.001), minimum temperature (R=0.254，P=0.001), maximum air pressure (R=0.313,P=0.000), minimum air pressure (R=0.305,P=0.000) and average air pressure (R=0.308,P=0.000), but the temperature difference and wind speed showed no significant correlation. The maximum humidity data displayed positive correlation with H1N1 onset no. (R=0.191,P=0.015). Our data did not exhibited correlation between hospitalized patients number and climatic factors (results not provided). After analysis of climatic factors of Harbin region and H1N1 onset no., no correlative climatic factors to H1N1 onset no. could be found in identical week, but climatic factors of identical week showed significant correlative to H1N1 onset no. of next week: maximum temperature (R=0.511, P=0.036), minimum temperature (R=0.515, P=0.035), maximum air pressure (R=0.566, P=0.018), minimum air pressure (R=0.598, P=0.011) and average air pressure (R=0.576, P=0.016).

Discussion

The 21st century is an era of influenza outbreaks. From the Spanish flu pandemic of 1918 to influenza pandemics of 1957 and 1968, the influenza lead to millions of deaths [7-8]. The outbreak of influenza A(H1N1) in 2009 showed widespread and strong infectivity, which resulted in global involvement. At present, the epidemic status of H1N1 has alleviated, but we still need more experiences and knowledge to investigate its epidemic characteristics. We speculated influenza A(H1N1) virus would display different disseminating features under different geographic conditions and climatic factors, thus all the data of H1N1 cases of Heilongjiang province in 2009 was analyzed and summarized in this study.

According to available data, we could found the susceptible peoples were the youths between 10-24 years old, similar to those in other countries and regions[9-11].Analyzed by ages segmented every 5 years, the peoples above 25 years old had an obviously low infective rate, the children below 5 years old and the aged between 60-64 years old had the lowest infective rates. The reason might be that the infants seldom contacted with the source of infection outside, and the aged have experienced the influenza pandemic in their youth and still kept a certain immunity. Early epidemic data from other countries also showed that older populations had a significantly lower probability of H1N1 infection[12].

The H1N1 outbreak has a characteristic of aggregation susceptibility, which results in more students were infected for their aggregation. The other

Table 3. Correlation between climate factors of every region and H1N1 onset No. of identical week in Heilongjiang province (17 weeks in all)

Climate factor	Onset no. of identical week in all regions		Onset no. of identical week in harbin region		Onset no. of next week in harbin region	
	Pearson Correlation	P (n=162▲)	Pearson Correlation	P (n=17)	Pearson Correlation	P (n=16)
Maximum temperature	0.262	0.001**	0.413	0.089	0.511	0.036*
Minimum temperature	0.254	0.001**	0.412	0.089	0.515	0.035*
Temperature difference	0.004	0.958	0.214	0.335	0.218	0.401
Maximum Air pressure	0.313	0.000**	0.395	0.105	0.566	0.018*
Minimum Air pressure	0.305	0.000**	0,375	0.125	0.598	0.011*
Average air pressure	0.308	0.000**	0.373	0.127	0.576	0.016*
Fastest wind speed	0.000	0.997	0.269	0.280	-0.081	0.758
Slowest wind speed	-0.053	0.501	-0.066	0.794	-0.002	0.993
Average wind speed	-0.036	0.649	0.129	0.610	-0.138	0.597
Average Humidity	0.046	0.560	-0.090	0.723	0.022	0.934
Maximum Humidity	0.191	0.015*	0.267	0.283	0.370	0.144

Significant correlation,P<0.05; **P<0.01. ▲, data on 0 H1N1 onset in each region were deleted.*

reason maybe they were too young to experience influenza pandemic and acquire immunity. Other studies have reported similar results[13-14]. The medical workers were seldom involved, maybe because of timely preventive and controlling training. One reason that the provincial capital had the highest onset numbers and high admission rates could be due to its highest population aggregation and mobility. Our data had not showed significant correlation between density of population and H1N1 onset no., perhaps due to small numbers of regions studied.

The feature that admission peak was 1 week later than the onset peak indicated the medical institution could provide essential preparation for the treatment. The onset date demonstrated the average time window from influenza symptoms to confirmed diagnosis is 4.1 days, but the average time window of admission was 5.4 days, which were slightly different to data of some previous reports[6,15,16], and might be correlative to different climatic conditions. Heilongjiang province lies in the north-east China, and is the northeast province of China., it locates at east longitude 128.75° and northern latitude 45.08°, a region in a ravine of mountain ridge. It is a cold-warm syndrome and damp-dry monsoon zone. The autumn and winter are the high-occurrence seasons (started from September and October) of respiratory diseases in Heilongjiang province. Therefore we analyze the correlation between climatic factors data and onsets and admission cases numbers alone. Interestingly, the temperature difference was not the decisive climatic factor for H1N1 prevalence. In Heilongjiang province, more H1N1 cases arose in areas of higher temperature and air pressure in autumn and winter. In some areas, the climatic factors of a certain week were correlative with the H1N1 onset of the next week, which might be the reason that the cases reported in the south China were more than those in north China in the same season. The results in our study did not reach a conclusion that the hospitalized patients number was relevant to local climatic factors. A considerate reason was that the hospitalized patients number was not enough, thus no significant statistical correlation could be obtained.

Our summary of these H1N1 data is of importance for the prevention and controlling of H1N1. Firstly, we would pay more attention to those susceptible populations before outbreak of influenza A(H1N1). Secondly, provincial capitals and students populations should be under intensive consideration. Thirdly, surveillance of climatic factors could facilitate the emergency disposal of H1N1 outbreaks. However, our study has some limitions. All the H1N1 cases underwent their laboratory detections in different city-level CDCs, which might lead to some undetected cases for the poor equipmental conditions or detection ability; some patients possibly did not go to local medical institutions because of slight influenza symptoms. Till now, few reports discussed the association between H1N1 and climatic factors, therefore it's difficult to carry out a horizontal equity and achieve an appropriate conclusion of their correlation.

In conclusion, through analysis of H1N1 data from Heilongjiang ,we got across the epidemic features of H1N1 outbreak in alpine-cold region, which suggesting a certain regularity. Meanwhile, our data showed that climatic factors might influence the outbreak and spreading of H1N1.

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Antimicrobial resistance of *Escherichia coli* isolated from the urine of outpatient clinic patients in the Zenica-Doboj Canton

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Abstract

Introduction: Urinary tract infections (IUTs) are considered to be the most common disease among adults, in particular women. IUTs are one of the most frequent reasons for prescribing antimicrobial therapy. *Escherichia coli* (*E. coli*) is the most frequent agent causing urinary infections not only in inpatients but also in outpatients. Urinary infection therapy is empirical and starts before its cause has been identified and its antimicrobial sensitivity/resistance determined. Having in mind the fact that *E. coli* accounts for 60–80% of IUTs, the empirical therapy of urinary infections includes implicitly the choice of an antibiotic which affects this bacterium. The aim of the study was to determine the antimicrobial resistance of *E. coli* isolated from the urine of outpatient clinic patients over a two-year period in the Zenica-Doboj Canton.

Material and methods: This paper is a retrospective analysis of urinocultures examined in 2011 and 2012. The microorganisms were identified using the standard microbiological method, whereas the bacteria sensitivity to eight tested antibiotics was examined using CLSI standards.

Results: The tested species showed the highest degree of resistance to amoxicillin (62.09%), sulfametoxazol-trimetroprim (45.47%), and ciprofloxacin (12.05%). The tested species of *E. coli* showed the lowest rate of resistance to nitrofurantoin (8.1%).

Conclusion: The monitoring of antimicrobial sensitivity/resistance to antibiotics of *E. coli* isolate, as a most frequent cause of IUTs, is very important not only for the proper prescription of antibiotics to treat IUTs, but also for the purpose of listing the first-rank medicines for their treatment.

Key words: Urinary tract infections (*IUTs*), *Escherichia coli* (*E. coli*), antimicrobial sensitivity/resistance.

Introduction

Urinary tract infections (*IUTs*) are considered to be the most common disease among adults, in particular women (1). Age, gender, and a variety of clinical syndromes increase the risk of urinary infections. Many of these infections are asymptomatic and do not require antimicrobial therapy (2). Statistical data indicate that 50% of women will have IUTs once in their lifetime, whereas 20–30% of women will have recurrent IUTs during their lifetime.

IUTs among men belong to the category of complicated urinary tract infections. They occur most frequently in men over the age of 50 and are connected with prostatitis syndrome. IUTs refer to infections of any part of the urinary system and differ according to their location, the presence or absence of symptoms, the required antimicrobial therapy, and the illness outcome. In epidemiological terms, IUTs are classified into inpatient infections, which mainly include the treatment with the urinary catheter, and outpatient infections (3). IUT diagnosis is based on clinical symptoms and laboratory findings of leukocyturia and bacteriuria. The best method of proving bacteriuria is the cultivation of a correctly taken urine sample and the existence of $\geq 10^5$ cfu/ml of urine (4). In etiological terms, the infection can be caused by various microorganisms. The most frequent agents causing IUTs are Gram-negative bacilli which belong to the *Enterobacteriaceae* family, and, in particular, *E. coli* (5). About 80% of IUTs in this study, especially among women, were caused by this bacterium (6). The therapy of IUTs starts before its causative agent has been identified and its antimicrobial sensitivity/resistance determined. A possible correction of a therapy initiated depends on clinical symptoms and microbiological analysis results, the presence of bacteria, and their

antimicrobial sensitivity. Having in mind the fact that *E. coli* accounts for 60–80% of IUTs, the empirical therapy of IUTs includes implicitly choosing the type of antibiotic that would affect this bacterium (7).

Material and Methods

In the Microbiology Ward of the Cantonal Hospital Zenica in the period between January 01, 2011 and December 12, 2012, all urine samples of outpatient clinic patients in the Zenica-Doboj Canton were analyzed. All samples were obtained using the midjet method, were cultivated on blood and McConky agar, and were incubated in aerobic conditions at 37^0C for 24 hours. *E. coli* was identified using standard biochemical tests. Samples whose number of the grown colonies was $\geq 10^5$ cfu/ml of urine were statistically analyzed. The antimicrobial sensitivity of all *E. coli* isolates was examined using the diffusion Kirby-Bauer method on Mueller-Hinton agar (Himedia, India)(8). The following antibiotics were tested: amoxicillin (10μg), cefasolin (30μg), cefuroxime (30μg), amoxicillin and clavulanic acid (10/20μg), gentamicin (10μg), ciprofloxacin (5μg), nitrofurantoin (300μg), and sulfametoxazol-trimetroprim (12.5/22.75μg) (Becton-Dicinson, USA). *E. coli* isolates isolated from the same patient over a two-month period with the same antimicrobial sensitivity were treated as duplicates and therefore were not statistically analyzed.

Results

Between January 01, 2011 and December 31, 2012, 52,271 urine samples from 20,858 patients of the Zenica-Doboj Canton were analyzed. 4,889 (9.35%) patients were identified as having significant bacteriuria and antimicrobial sensitivity/resistance. Table 1.

Table 1. Distribution of positive and negative urinocultures from outpatient clinic patients in the Zenica-Doboj Canton

Patients	Number	Percentage %
Positive urinocultures with significant bacteruria	4,889.00	9.35
Negative urinocultures	47,382.00	90.65
Total	52,271.00	100.00

Out of the overall number of patients with positive urinocultures, *E. coli* was identified in 2,794 (57.15%) patients. Table 2.

Table 2. Presence of E. coli isolates in the patients with positive urinocultures

Patients with significant bacteriuria	Number	Percentage %
E.coli	2,794.00	57.15
Other	2,095.00	42.85
Total	4,889.00	100.00

Table 3. Antimicrobial resistance of E. coli species isolated from the urine of outpatient clinic patients between the years 2011 and 2012

Tested antibiotics	R (number)	R %	S (number)	S %	I (number)	I %	Number of tested antibiotics
AMX	1007	62.90	557	34.79	37	2.31	1601
AMC	311	11.22	2421	87.34	40	1.44	2772
CZ	161	14.87	901	83.19	21	1.94	1083
CXM	256	9.42	2440	89.74	23	0.85	2719
CIP	245	12.05	1782	87.61	7	0.34	2034
GM	297	11.32	2296	87.53	30	1.14	2623
SXT	1189	45.47	1405	53.73	21	0.80	2615
F/M	214	8.01	2415	90.45	41	1.54	2670

AMX = amoxicillin; AMC = amoxicillin and clavulanic acid; CZ = cefasolin; CXM = cefuroxime; GEN = gentamicin; CIP = ciprofloxacin; SXT = sulfametoxazol trimetroprim; F/M = nitrofurantoin

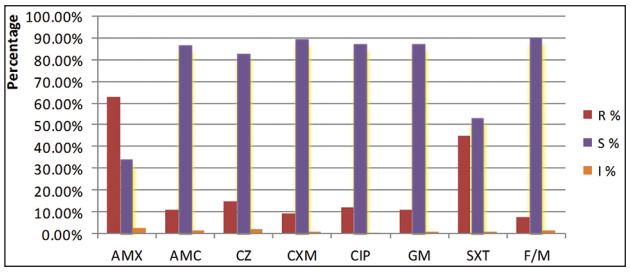

Chart 1. Antimicrobial sensitivity/resistance of E. coli species isolated from outpatient in the Zenica-Doboj Canton between the years 2011 and 2012.

Discussion

Being well acquainted with the resistance of bacteria to antimicrobial medicines is the main prerequisite for successful bacterial infection therapy. IUTs are considered to be the most frequent infection among adults, in particular women. This is one of the main reasons for prescribing antibiotics not only in the treatment of acute and chronic infections, but also for prophylactic purposes. IUT therapy is mainly empirical. In the case of uncomplicated IUTs, the use of antibiotics without a previous microbiological analysis is justifiable if it has been observed which bacterial species are the most frequent causal agents of the infection and what their resistance is at the local level. *E. coli* dominates worldwide as an etiological cause of complicated and uncomplicated urinary tract infections (2, 3, 4). Until recently *E. coli* was considered to be a bacterium very much susceptible to a wide range of antibiotics. However, it has been frequently observed that there has been an increase in *E. coli* species resistant to a number of antibiotics. It has also been observed that the *E. coli* species is increasingly resistant to ampicillin and sulfametoxazol-trimetropin, and that the *E. coli* species has secreted Extended Spectrum Beta-Lactamase (ESBL) (9). Urinary infections caused by multiple-resistant *E. coli* species pose a clinical problem because of a limited number of therapeutic options (10).

This paper deals with the antimicrobial resistance of *E. coli* species isolated from outpatient clinic patients in the Zenica-Doboj Canton in 2011 and 2012. A relatively low percentage (9.34%) of isolates with identified „significant bacteriuria“ (positive urinocultures) has been observed. The reason for it may be found in a nonselective testing of samples (11). *E. coli* was isolated from 57.15% positive samples, which is in accordance with the data found in the literature (12). During the examination period, *E. coli* species isolated from the urine of Zenica-Doboj Canton patients showed the highest resistance to amoxicillin (62.9%) and sulfametoxazol-trimetroprim (45.47%).

Resistance to these two antibiotics recorded in the Zenica-Doboj Canton are higher in comparison with the average resistance recorded in the Republic of Croatia (49% and 24% respectively) and in some European countries (13.14%). The results of *E. coli* resistance to these antibiotics may be compared to the results in the Fojnica area found in the 2011 Report (6) and among previous examination results in this Canton (10, 11, 12). The reason behind such a high resistance level may be found in the fact that these antibiotics had been for a long time first-choice medicines in IUT treatment, whereas sulfametoxazol-trimetroprim had been a choice medicine for urinary infection prophylaxis.

The resistance to gentamicin (11.32%) was considerably higher than the resistance in the Primorsko-Goranjska Canton, the Republic of Croatia, for the period between the years 2003 and 2008, which ranged from 2.22% to 6.28% respectively (15). *E. coli* resistance to gentamicin in Europe amounts to 2% on average and is lower in comparison to our investigation results (14). The isolated *E. coli* species according to our investigation showed 12.05% resistance to ciprofloxacin. Such a high resistance level is worrying because, according to the recommendations of the Infectious Diseases Society of America (ISDA), ciprofloxacin is a first-choice medicine for IUT treatment in media where resistance to sulfametoxazol-trimetroprim > 20% (16). Our data concerning the resistance of the *E. coli* isolated species to ciprofloxacin depart considerably from the investigation data registered in the Fojnica area (0.8%) (6). Such a high level of resistance to ciprofloxacin was registered in the Republic of Croatia, Hong Kong, and the USA (13%, 13.9%, and 25% respectively) (13, 14, 17, 18). It was found to occur with older patients and those with frequent IUTs. One of the reasons for such a high level of *E. coli* resistance to all tested antibiotics in the Zenica-Doboj Canton is the fact that these antibiotics were allowed to be bought without a doctor's prescription (11).

According to our investigation, isolated *E. coli* species are found to show the lowest level of resistance to nitrofurantoin (8.1%), so that it can be recommended as a first-choice medicine in IUT treatment. Still, although *E. coli* showed the lowest level of resistance to nitrofurantoin in our investigation, it is much higher in comparison with the average in the Republic of Croatia (4%) and European countries (0.7 – 3%) (13,14).

A medicine that should be tested is fosfomycin thrometamine, with which, so far, no higher level of resistance has been registered in Europe (19, 20). In statistical terms, the age of patients with whom *E. coli* resistant species were isolated should be routinely analyzed, which would additionally rationalize prescription of antimicrobial therapy.

Conclusion

The monitoring of antimicrobial sensitivity/ resistance to antibiotics of *E. coli* isolate, as the most frequent cause of IUTs, is important so that antibiotics can be rationally prescribed and so that they can be listed as first-rank medicines for healing IUTs.

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A study of the relationshop between sociotropy-autonomy personality traits and levels of problem solving and coping with stress in nursing students

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Abstract

Aim: The purpose of this study was to determine the relationship between the sociotropy-autonomy personality traits of student nurses and the ways in which they solved problems and coped with stress.

Method: This descriptive study was carried out in February 2013. Data collection was performed using an Identification Information Form (IIF) developed by the researchers, the Sociotropy-Autonomy Scale (SAC), the Problem-Solving Inventory (PSI), and the Coping Strategies Scale (CSS). The study was carried out on 125 first and second year students at a university nursing department. In evaluation of the data, descriptive statistics and correlation analysis were used.

Findings: It was determined that 87.2% of the student nurses were female and that their mean age was 19.26 ± 0.86 years; 64% of the students were in their first year; 79.2% had gained a place in nursing in their first year; 58.4% had chosen it voluntarily, and it was among the first three choices for 40.8%. Mean scores were 64.94 ± 15.63 *(max=120)* for sociotropy, 72.15 ± 1.48 *(max=120)* for autonomy, and 89.52 ± 1.80 *(max=192)* for PSI, and mean scores on CSS subscales were $CSS_{self\text{-}reliance}$ = 14.36 ± 3.128 *(max=21)*, $CSS_{desperation}$ = 11.11±3.93 *(max=24)*, $CSS_{submissiveness}$ = 5.96±3.21 *(max=18)*, $CSS_{optimism}$ = 9.44±2.18 *(max=15)* and $CSS_{seeking\ social\ support}$ = 7.64±2.10 *(max=12)*. A relationship at a statistically significant level was found between mean sociotropy-autonomy scores and the mean scores on the subscales of problem-solving and coping with stress ($p<0.01$, $p<0.05$).

Results: It was found that autonomic (independence) personality traits rather than sociotropic (social belonging) traits were dominant among the nursing students; they perceived themselves as adequate in terms of problem-solving, and there was a relationship between their sociotropy-autonomy personality traits and their methods of problem solving and coping with stress.

Key words: Autonomy, sociotropy, problem solving, coping with stress, nursing student, personality traits.

Introduction

Nurses are part of the basic foundations of the health system, but as this system increases in complexity they face constant problems and therefore experience greater stress. They face many risk factors which may cause stress, such as long working hours, an excessive work load, time pressure, difficult or complex duties, insufficient rest, monotony, poor physical work conditions (such as location, heat and lighting), long periods standing up, lack of sleep when in night duty, and irregular eating patterns (1). In addition, the fact that this profession is largely occupied by women adds a further dimension to the levels of stress. The traditional Turkish family has a patriarchal structure, so that problems arising from a woman's working life come on top of the problems and social pressures of her home life as a woman and a mother (2). This stress experienced by nurses in the long term causes fatigue and a reduction in motivation and job satisfaction (3), and can have an adverse effect on nurse-patient communication and interaction. For this reason the coping mechanisms which nurses use or could potentially use are of great importance for nurse-patient interaction and the care services which nurses provide. Identifying the methods which nurses use to cope with stress can contribute to creating an efficient care model for patients and thus improving the process of interaction between nurses and patients (4, 5, 6).

The stress experienced by nurses in their working environment is as much an institutional problem as it is an individual one inasmuch as it affects the quality of the care services provided. The ability of nurses to provide reliable and quality care depends on their capacity to cope with stress and on their problem-solving skills. Therefore, it is expected that these qualities should be found at a high level in nurses (7). Coping with stress and problem solving skills are affected as much by nurses' past experience, perceptive strength, environment, and their attitudes and skills in relation to cultural factors and events as by personal characteristics (4, 5, 6).

In Beck's cognitive theory as set out by Kabakçı, two personality dimensions are involved in dependence on others or self-reliance. These are sociotropy (social belonging) and autonomy (independence). Sociotropy is characterised by a person's need for positive relationships with other people, while autonomy is shown by a person's independence and need to achieve certain goals. For highly sociotropic individuals, it is of great importance to obtain the approval of others, while those with a high degree of autonomy place great importance on personal success or failure. Stress and various psychological problems can be caused in individuals in whom sociotropic characteristics are dominant by perceived loss or rejection in interpersonal relations; for people in whom autonomic characteristics are dominant however, these problems can be caused by a loss of control over their environment or a perceived failure (8).

According to Rosenbaum (1989), effective coping with stress is possible with the ability to control internal and external events, in other words with autonomy (9). Individual autonomy, which is a reflection of professional autonomy, is of the greatest importance in the nursing profession in that it is basic in gaining professional status (10). Autonomy provides a nurse with self-identification, independence and the ability to share difficulties (8, 11). In care giving, it enables service to be provided in a purposeful and controlled manner, keeping it under the control of outside forces rather than leaving identification of the role of nurses and nursing practice to chance. In addition, a deliberate decision process is brought into operation, enabling care strategies to be developed and responsibility to be taken for the exercise of authority (12). Autonomy enables nurses to take responsibility when they encounter problems in providing care, to see themselves as competent to solve problems and to develop new approaches to new situations (11, 13, 14). Also, it charges nurses with defining their own future and taking responsibility for their mistakes (15). Sociotropy on the other hand contributes to professionalization by causing dependence on the profession (11, 13, 16, 17). In coping with stress and problem solving, nurses will tend to act in accordance with the decision of the team rather on their own account (13). In this connection, there is a need to determine what effect sociotropy-autonomy personality traits have on coping with stress and on decision making, and what kind of relationship there is between them. Studies exist in the literature showing the relationship between personality traits and coping with stress (11, 18), but insufficient studies were found on the relationship between the personality traits of student nurses and their methods of coping with stress and problem solving skills. The aim of the present study was to determine the sociotropy-autonomy personality traits of nursing students, and the relationship between these traits and their methods of coping with stress and their problem solving skills. One important outcome of nursing training is the development of skills to cope with stress and to solve problems. In this regard the results of the study will be expected to contribute to new dimensions in nursing training. In addition, it is thought that it can play a leading role in the future in programmes for nurses to develop methods of coping with stress and problem solving.

Materials and methods

This descriptive study was carried out in February 2013 with 216 first and second year nursing students in the academic year 2012-2013 in the Nursing Department of the Health Sciences Faculty of a university. There was no selection of the sample, and the research was conducted on 125 student volunteers. The participation rate was 57.9%; 64.0% of the participants were in their first year, and 36.0% were second year students. An Identification Information Form (IIF) developed by the researchers and three scales were used in the collection of data.

Identification Information Form (IIF)

This form, prepared after an examination of the literature, consisted of six open-ended questions and 14 closed questions. These 20 questions were designed to elicit

* the nurses' sociodemographic characteristics (e.g. age, gender, high school education, place lived in for the longest time, place of residence, education level of parents, family type)
* information on their choice of nursing (e.g. whether nursing was a deliberate choice, order of preference, year of entry to the course, academic average)
* their perceptions relating to stress experienced during clinical practice, their independence, and their skills in coping with stress and solving problems.

Sociotropy-Autonomy Scale (SAC)

This scale measures dependence and self-reliance character traits. It was developed by Beck et al. (1983) and adapted for Turkish and tested for validity and reliability by Şahin et al. (1993). It consists of 60 items, 30 on sociotropy and 30 on autonomy. Each item has five Likert-type responses scored from 0 to 4, thus: "Does not describe at all-0"; "Describes somewhat-1"; "Describes quite well-2"; "Describes well-3"; "Describes very well-4". The total score for each subsection is 0-120. The subsections of the scale are evaluated separately and there is no total score for the scale. A high score indicates the personality traits of that subsection (19). For the original form, the test-retest reliability coefficient was found to be between α.65 and α.88 for the sociotropy trait and between α.66 and α.75 for the trait of autonomy, while the alpha reliability coefficient of the Turkish form was α.70 for sociotropy and α.81 for autonomy (19). In the present study, the Cronbach alpha reliability coefficient of the sociotropy subsection of the scale was found to be 0.74, and that of the autonomy subsection was 0.77.

Problem Solving Inventory (PSI)

This was devised by Heppner and Petersen (1982) to measure the perception of individuals with regard to their problem solving skills (20). Turkish validity and reliability was carried out by Şahin et al. (1993)(19). The scale consists of 35 items applicable to adolescents and adults and is reliant on self-reporting. Participants rate each item on a six-point Likert-type scale ranging from "I always act that way-1" to "I never act that way-6". In evaluating the scale items, items 9, 22 and 29 were not scored. Items 1, 2, 3, 4, 13, 14, 15, 17, 21, 25, 26, 30 and 32 were scored the opposite way round. These items were taken to represent sufficient problem solving skills. The range of scoring in the scale was 32-192. High points showed that an individual perceived him/herself as inadequate in terms of problem solving skills.

Ways of Coping Inventory (WCI)

The Ways of Coping Inventory was devised in 1980 by Folkman and Lazarus to show the ways individuals coped with general or specific situations of stress. It consists of 30 items. According to Tuğrul, the first standardisation work on this scale in Turkey was carried out by Siva (1991). The scale has five dimensions: Self-confident approach (8, 10, 14, 16, 20, 23, 26), Desperate approach (3, 7, 11, 19, 22, 25, 27, 28), Submissive approach (5, 13, 15, 17, 21, 24), Optimistic approach (2, 4, 6, 12, 18), and the Social support-seeking approach (1, 9, 29, 30) (5). In evaluating the scale, higher scores on the dimensions of self-reliance, optimism and social support seeking indicate effective coping, whereas higher scores on the dimensions of desperation and submissiveness show the use of ineffective methods of coping (22, 23).

Cronbach Alpha reliability coefficients of the subscales obtained by factor analysis in a study of the CSS performing psychometric evaluation by Şahin et al. were found to be as follows: Optimistic approach 68, Self-Reliant approach 80, Desperate approach 73, submissive approach 70, Social support seeking 47. Responses to the items on the scale were in four steps from "not at all applicable" to "very applicable". Items 1 and 9 were scored the other way round. Total points were not counted, but each subscale was calculated separately (21).

Oral informed consent was obtained from the nursing students to participate in the study. Descriptive statistics were used in the evaluation of the study findings, and correlation analysis was used to compare data. Results were evaluated with a

95% confidence interval, and $p<0.05$ was taken as the level of significance.

Findings

It was found that 87.2% of the nursing students were female and their mean age was 19.26 ± 0.86 years; 52.0% were graduates of Anatolian High Schools and 38.4% were from normal high schools; 64.0% were in their first year of study. It was determined that 79.2% of the students had gained a place in Nursing in their first year, 58.4% had chosen voluntarily, and it was among the first three choices of 42.4%. The nursing students characterised themselves in this way using a multiple choice system (Table 1):

Table 1. Distribution of nursing students according to their own self-characterisation

	No	%
Hasty	43	34.4
Attentive to details	78	**62.4**
Shy	44	35.2
Self-reliant	53	**42.4**
Well-planned	40	32.0
Rationel	54	**43.2**
Intuitional	35	28.0
Dependent	6	4.8
Spontaneous	9	7.2
Evaluative	52	**41.6**

48.0% of the nursing students stated that they would feel the need to seek the approval of clinical nurses when carrying out care of a patient. 40.0% stated that they would feel partly independent when carrying out a procedure, 9.6% stated that they would not feel independent, and 48.8% did not answer the question. 32.8% stated that they experienced stress when carrying out clinical procedures, the reasons for which are set out in Table 2.

When the nursing students were asked whether they were independent in caring for patients, 62.8% stated that they could be independent. When asked "Should nursing students have the skill to take responsibility and solve problems?" 84.8% answered that they should have the skill to take responsibility and 93.6% stated that they should have problem solving skills. When asked about their own level of development of the skills of problem solving and coping with stress, 28.8% of the students said that their skill in taking responsibility was at a medium level and 57.6% that it was at a high level; 55.2% rated their problem solving skills as medium 24.8% rated them as high; and 42.2% stated that their stress coping skills were medium and 16.8% that they were high.

The nursing students' mean scores were 64.94±15.63 *(max=120)* for sociotropy, for 72.15±1.48 *(max=120)* autonomy, and 89.52±1.80 *(max=192)* on PSI; their mean scores on the CSS subscales were $CSS_{self\text{-}reliance}$ = 14.36 ± 3.128 *(max=21)*, $CSS_{desperation}$ = 11.11±3.93 *(max=24)*, $CSS_{submissiveness}$ = 5.96±3.21 *(max=18)*, $CSS_{optimism}$ = 9.44±2.18 *(max=15)* and $CSS_{seeking\ social\ support}$ = 7.64±2.10 *(max=12*.

A statistically significant direct relationship was found between mean sociotropy-autonomy scores and the mean scores of the PCI and CSS subscales. Analysis showed that there were direct relationships between the mean sociotropy score and the means scores of PCI and the $CSS_{desperation}$ subscale and between the mean autonomy score and those of the $CSS_{self\text{-}reliance}$ and $CSS_{optimism}$ sub-

Table 2. Distribution of nursing students according to reasons for stress

	No	%
Uncertainty about the responsibilities expected of students	16	12.8
Conflicts with members of the team	12	9.6
Inadequate conditions of the physical environment (constricted workspace)	8	6.4
Inadequate equipment	3	2.4
Excessive workload	4	3.2
Conflict with patients' relatives	2	1.6
Dissatisfaction with the care offered to patients	4	3.2
Inadequate support given by teaching staff	3	2.4
Inadequate rewards	1	0.8

Table 3. The relationship between mean sociotropy-autonomy scores and mean problem solving and coping subscale scores

Direct Relationship	Inverse Relationship
➢ Sociotropy → PSI $_{(p< 0.01)}$	* Autonomy X PSI $_{(p< 0.01)}$
➢ Sociotropy → $CSS_{desperation}$ $(p< 0.01)$	* Sociotropy X $CSS_{optimism}$ $(p< 0.01)$
➢ Autonomy → $CSS_{self\text{-}reliance}$ $(p< 0.01)$	* Autonomy X $CSS_{desperation}$ $(p< 0.01)$
➢ Autonomy → $CSS_{optimism}$ $(p< 0.05)$	* Autonomy X $CSS_{seeking\ social\ support}$ $(p< 0.05)$
➢ PSI → $CSS_{desperation}$ $(p< 0.01)$	* PSI X $CSS_{self\text{-}reliance}$ $(p< 0.01)$
➢ PSI → $CSS_{submissiveness}$ $(p< 0.01)$	* PSI X $CSS_{optimism}$ $(p< 0.01)$

scales, while there was an inverse relationship between mean sociotropy scores and the mean score of the $CSS_{optimism}$ subscale and between the mean autonomy score and mean PCI, $CSS_{desperation}$ and $CSS_{seeking\ social\ support}$ subscale scores ($p<0.01$, $p<0.05$) (Table 3).

Discussion

Unlike in previous years (11), it can be seen that more than half of the students in the present study chose the nursing profession on their own initiative. This may be because the assurance of finding a job after obtaining a nursing degree has increased recently, while the public image and perception of nursing has also improved.

It was shown that the nursing students' PSI mean scores were at a medium level or in other words that they felt that their problem solving skills were adequate. Similarly, when students were asked to characterise themselves, 42.4% stated that they were self-reliant. The nurses' perception of themselves as adequate at individual problem solving and as self-reliant means that they are aware of problems relating to family and social well-being, they are ready to create solutions, and they have the skill to translate these solutions into action. Moreover, this is an indication that the nursing student can take on the responsibility to preserve life. Most importantly, this means bringing effectiveness, efficiency and quality of service, change, professionalism, autonomy and strength (24). Along with all of this, having adequate problem solving skills helps flexibility and adaptability and the ability to develop suitable methods to achieve a goal. This will further help to reduce the effects of personal and interpersonal problems and serve as a mechanism of coping and reducing the negative effects of stress. Thus, it can be seen in our results that there was a statistically significant relationship between the mean PSI and CSS scores (Table 3).

A third of the nursing students reported experiencing stress during clinical practice, and gave the most basic reason for this as "the responsibilities of students being undefined." It was shown that they tended to use the approaches of self-reliance, optimism and seeking social support rather than submissiveness or desperation in coping with stress. Some coping methods cause negative feelings towards stress, and while a protective action that produces alternative solutions may help, some make the effects of stress worse and may lead to problems in accommodating (25, 26). It was seen that the students chose more constructive approaches as protective actions to cope with stress.

Almost all of the nursing students believed that it was necessary to have the skills of taking responsibility (27) and problem solving, which were a basic condition for autonomy, in the care of patients. Also, a large number stated that their own skills of taking responsibility, coping with stress and problem solving were above the medium level. However, almost half of the students stated that when on practice they felt the need to seek the approval of clinical nurses when carrying out patient care, and that they could not be completely independent. Similarly, Weis and Schank (1997) found a relationship between career development of student nurses in the United States and autonomy and independence, and reported that in a study by Beeman on

British nursing students the students were given responsibility for patient care, but they did not have a voice in care-related decisions and they had very little autonomy in that respect (28). In spite of all this, it was observed that the students' autonomic personality traits were more dominant than their sociotropic traits. In a study of 369 nursing students, it was similarly reported that the students' autonomic personality traits (77.25 ± *14.45) were more dominant (11).* In a further study carried out on nurses, it was found that the nurses' mean scores on the autonomic scale (75.95 ± 16.22) were higher (29). Autonomy has an important role in individuals' self-identification, enthusiasm for their profession and meeting professional standards (16, 30). The fact that at this point the autonomic personality traits of the nurses of the future are dominant is a hopeful sign for the future image and perception of the nursing profession.

It was established that there was a statistically significant relationship between the nursing students' sociotropy-autonomy personality traits and their problem solving and coping strategies. As the students' tendency towards sociotropic personality traits increased, their tendency to feel inadequate with regard to problem solving also increased, and they showed a greater tendency towards the desperate and submissive approaches in coping with stress. In contrast, as the tendency to autonomic personality traits increased, they perceived themselves as adequate in problem solving, and tended towards the seeking social support, self-reliant and optimistic approaches in coping with stress. This finding is noteworthy in showing the need for developing autonomic personality traits rather than sociotropic traits in nursing training (31). Autonomy necessitates decision making, authority, and taking responsibility, and means strength (31). Individual autonomy is basic to establishing professional status and so is of the greatest importance in the nursing profession (10) and develops inside supportive social relationships and structures (31, 32, 33, 34).

Conclusion

It was established that the nursing students' autonomic personality traits were more dominant than their sociotropic traits, that they perceived themselves to be adequate in terms of problem solving, and that they showed a tendency to use constructive approaches (self-reliance, optimism and seeking social support) in coping with stressful situations. In addition, it was established that as the students' autonomic personality traits developed, they perceived themselves as more capable of problem solving and showed a tendency to use more constructive approaches (optimism, self-reliance and seeking social support) in coping with stress. In view of these results, it is recommended that measures be taken to include the development of autonomic personality traits in nursing education, whether in theoretical or in-service training, and especially to accord a place to knowledge and skills that will enable nurses to feel adequate and equipped to solve problems and cope with stress. It is also recommended that students should be given an awareness of their own personality traits and the relation between these and their coping and problem solving skills. It is felt that this will contribute to the establishment of more effective and suitable strategies for nursing students to cope with stress and to solve problems.

One limitation of this study was that it was conducted on a nursing degree programme which was newly opened and therefore had only first and second-year students. For this reason it is recommended that a similar study should be carried out on a wider sample including those in higher classes who have taken classes in other nursing topics.

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Gram staining for the morphological identification of cytolytic vaginosis versus vulvovaginal candidiasis

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Abstract

Background: Cytolytic vaginosis (CV) is a syndrome caused by overgrowth of *lactobacillus*. With the symptoms of pruritus, dyspareunia and whitish-cheesy vaginal discharge, it is often misdiagnosed as vulvovaginal candidiasis (VVC). Since the symptoms of CV resemble those of VVC, differentiating it from candidiasis under the microscope is especially important.

Methods: 108 subjects were included in the morphological study, including 21 healthy women, 33 patients with CV and 54 patients with VVC. Vaginal discharge were collected and made into smears. After gram staining, the morphological characteristics of these 108 vaginal smears were observed under the microscope. The smears were assessed for the quantity of lactobacilli, epithelial cell morphology and the absence or presence of *Candida* species, *Trichomonas vaginalis* and clue sells.

Results: The morphological characteristic of patients with CV consisting of overgrowth of lactobacilli, the presence of naked nuclei and fragments of the epithelial cells, a paucity of leukocytes, the absence of *Candida* species and other pathogens. However, the morphological characteristic of patients with VVC consisting of the presence or absence of lactobacilli, the presence of normal epithelial cells, candidal spores, blastospores, hyphae or other pathogens **such as** *T.vaginalis* and *Gardnerella vaginalis.*

Conclusion: CV and VVC can be identified based on the quantity of lactobacilli, epithelial cell morphology and the absence or presence of *Candida* species and other pathogens and the misdiagnosis of CV as VVC can be avoided.

Key words: Cytolytic vaginosis, *Lactobacilli*, Epithelial cell, Vulvovaginal candidiasis, Morphology.

Introduction

Doderlein[1] first described the normal vaginal flora as predominantly consisting of acid-producing gram-positive *Lactobacillus* rods. Healthy women of reproductive age are usually colonized by *Lactobacillus* and *Gardnerella vaginalis*.[2, 3] Some *Lactobacillus* species produce hydrogen peroxide (H_2O_2) and lactic acid, which is toxic to various microorganisms. This phenomenon may prevent overgrowth of organisms such as *Escherichia coli*, *Candida species*, *G. vaginalis* and *Mobilincus* species.[2, 4, 5] According to several studies, *lactobacilli* create a barrier against candidal overgrowth by blocking the adhesion of yeast to vaginal epithelial cells through competitive feeding.[6] These studies indicated an antagonistic relationship between *Lactobacillus* and *Candida species.*

A small proportion of women of reproductive age may have l*actobacilli overgrowth*. In these patients, *lactobacilli* alone or in combination with other bacteria may damage the vaginal intermediate epithelium and may result in cell dissolution, leading to a disease called cytolytic vaginosis (CV) with the symptoms of pruritus, dyspareunia, vulvar dysuria and profuse vaginal discharge, especially whitish-cheesy vaginal discharge.[7] These individuals are often misdiagnosed with candidiasis, as evidenced by their lack of response to repeated antifungal therapy regimens for suspected recurrent vulvovaginal candidiasis.[8] It has also been observed that symptoms will be more severe during the luteal phase, in which it has been suggested that there is a remarkable rise in the number of colonizing *lactobacilli that can lead to the cyclical occurrence of* CV.[6, 7, 9, 10]

Many studies have indicated that special attention must be given during the evaluation of vaginal smears of patients with presumed vaginal candidiasis to prevent a misdiagnosis of CV. In one study

of 271 patients with vulvovaginal complaints, 29 (10.7%) were diagnosed with suggestive vulvovaginal candidiasis (VVC), but only 16 (5.9%) had a confirmed diagnosis of candidiasis. Although not mentioned in the article, the remaining cases could have been diagnosed as CV if further investigations had been carried out.[11] By examining 2,947 Papanicolaou-stained vaginal smears, Demirezen S[12] found that 54 of 2,947 patients (1.83%) were diagnosed with CV based on cytological criteria. Batashki I et al[13] evaluated the frequency of CV among women with symptoms that mimic VVC. Among 1,152 patients who were microbiologically and cytologically tested, 3.9% had the clinical presentation of CV that was suggestive of VVC. The differentiation of these 2 conditions is essential to the appropriate treatment and resolution of chronic subjective vaginal complaints.[14]

Several epidemiological surveys of CV have been conducted in the North American and European regions of Turkey, Sweden, USA and Bulgaria.[11-13, 15, 16] The diagnosis of CV was mainly based on the diagnostic criteria suggested by Cibley LJ.[7] However, the morphological identification between CV and VVC is often overlooked by clinicians, and few studies have examined the issue in developing and developed countries. In addition, little has been known about their morbidity and morphological identifications in developing countries such as China.[17, 18] Clinicians in China often overlook the cause of cyclic vaginal itching and burning and misdiagnose it as VVC. Despite energetic propaganda, the diagnosis of CV has been established in few hospitals in China.

Since the symptoms of CV resemble those of VVC, differentiating it from candidiasis under the microscope is especially important. This study aimed to identify the morphological characteristic of vaginal discharge in healthy women, patients with CV and patients with VVC to avoid confusion of the 3 groups by clinicians and prevent the overlooking or misdiagnosis of CV.

Subjects and methods

The study was conducted in October 2012 in the gynecology and obstetrics department of West China Second University Hospital, Sichuan University, China. Morphological observations were performed in the department of laboratory medicine of the same hospital. All procedures and protocols followed in the study received prior approval from the ethics committee of this unit (Medical Research 2012 Application No.13). Informed consent was obtained from all participants.

108 subjects enrolled in the morphological study, including 21 healthy women with normal vaginal discharge; 18 with mild CV and 15 with severe CV; 20 with single infectious VVC; 16 with VVC combined with trichomoniasis and 18 with VVC combined with bacterial vaginosis（BV）. Participants were asked about their ages, pregnancy, education, marital Status, symptoms, the nature of their complaints (color， amount of discharge and presence of itching, pruritus, dyspareunia or vulvar dysuria), past illness, and history of treatment before undergoing gynecological examination. HIV infected, and menstruating women or those who had used antibiotics and/or topical vaginal creams within seven days prior to the date of examination were specifically excluded from the study.

Vaginal discharge samples of the 108 subjects were collected on dry sterile cotton wool-tipped swabs (Medical Apparatus and Instruments Factory of Yangzhou Chuangxin, Jiangsu, China), made into smears and sent to the Department of Laboratory Medicine, West China Second University Hospital, Sichuan University for examination.

Firstly, the *T. vaginalis* was observed under the low power field of microscope (OLYMPUS, Tokyo, Japan) with wet smears; then, the smears were fixed by a slide heater (WESCOR, Utah, USA) for 1 min, stained with crystal violet for 10 s, washed in tap water, and flooded with iodine for 6-10 s. they were then washed in tap water several times, decolorized with acetone–alcohol, and finally counterstained with safranin for 10 s. After rinsing with tap water, the slide was left in air or a slide heater to dry before microscopy.

After gram staining, the morphological characteristics of the vaginal smears including *lactobacilli, e*pithelial cells, *Candida* species, *T. vaginalis* (verifying the results of wet smears) and clue cells were observed under oil immersion field of microscope by laboratory microbiologists.[19] The laboratory followed the guidelines of both the National System of External Assessment of the Quality and the College of American Pathologists.

The diagnosis of CV was mainly based on the diagnostic criteria suggested by Cibley LJ.[7] The criterion for diagnosis of BV was based on Gram-stained smear as described previously [20-22]. The diagnoses of VVC and trichomoniasis were based on spore or pseudohyphae and Trichomonas vaginalis, respectively, identified by microscopic examination.

All data analyses were performed by SPSS 17.0 (Chicago, USA). The descriptive statistics, the t-test and the chi-square test were used to identify significant differences of age, pregnancy, education and marital Status between patients with CV and healthy women, patients with CV and patients with VVC, patients with VVC and healthy women. A two-tailed p-value < 0.05 was considered statistically significant in all of the statistical analyses.

Results

Nineteen women were excluded, and the 108 women enrolled in the study ranged from 18 to 64 years old (mean, 36 years). Table 1 shows the demographic characteristics of the women enrolled. They were primarily middle-aged females (mean age 36.7 ± 11.34 years). The mean age of patients with CV(30.7±8.52) was lower than the healthy women(35.2±10.05) and the patients with VVC (37.8±12.42), the differences were statistically significant (t-test showing p=0.042 and 0.039, respectively). The pregnant rate of patients with CV (81.8%) was higher than that of the healthy women and the patients with VVC, the differences were also statistically significant (chi-square test showing p=0.012 and 0.024, respectively). Marital Status of the three sub-group did not give a statistically significant difference (chi-square test showing

Table 1. Demographic profile of the subjects enrolled in this study along with the patients with CV, the healthy women and the patients with VVC

Characteristics	Study subjects (n=108)	Healthy women (n=21)	Patients with CV (n=33)	Patients with VVC (n=54)
Age at presentation *(years)				
Mean±SD	36.7±11.34	35.2±10.05	30.7±8.52	37.8±12.42
Median(range)	36(18-64)	35(21-53)	30(18-42)	37.5(20-64)
Pregnancy§				
not pregnant	43(39.8%)	19(90.5%)	6(18.2%)	38(70.4%)
Currently pregnant	65(60.2%)	2(9.5%)	27(81.8%)	16(29.6%)
Marital Status†				
Single	3(2.8%)	1(4.8%)	2(6.1%)	0(0%)
Currently Married	96(88.9%)	19(90.4%)	31(93.9%)	46(85.2%)
Widowed/ Divorced/separated	9(8.3%)	1(4.8%)	0(0%)	8(14.8%)
Education‡				
Elementary	15(13.9%)	3(14.3%)	3(9.1%)	8(14.8%)
High school	65(60.2)	8(38.1%)	14(42.4%)	35(64.8%)
Graduate	28(25.9%)	10(47.6%)	16(48.5%)	11(20.4%)

** t-test between patients with CV and healthy women sub-group for age at presentation p = 0.042 (t= 2.158); t-test between patients with CV and patients with VVC sub-group for age p = 0.039 (t= 2.313); t-test between patients with VVC and healthy women sub-group for age at presentation p = 0.316 (t= 0.732)*

§ Chi-square between patients with CV and healthy women sub-group for pregnancy p = 0.012 (chi-square=9.13, df =1); Chi-square between patients with CV and patients with VVC sub-group for pregnancy p = 0.024 (chi-square =7.41, df=1); Chi-square between patients with VVC and healthy women sub-group for pregnancy p = 0.108 (chi-square =4.51, df=1)

†Chi-square between patients with CV and healthy women sub-group for marital status p = 0.248 (chi-square =2.78, df=2); Chi-square between patients with CV and patients with VVC sub-group for marital status p = 0.057 (chi-square=5.79, df=2); Chi-square between patients with VVC and healthy women sub-group for marital status p = 0.412 (chi-square = 1.53, df=2)

‡ Chi-square between patients with CV and healthy women sub-group for education p = 0.482 (chi-square =1.468, df=2); Chi-square between patients with CV and patients with VVC sub-group for education p = 0.048 (chi-square = 6.23, df =2); Chi-square between patients with VVC and healthy women sub-group for education p = 0.043 (chi-square = 6.52, df =2)

p=0.248, 0.057 and 0.412, respectively). However, the education status of patients with VVC was different significantly with the patients with CV and the healthy women sub-group (chi-square test showing p=0.048 and 0.043, respectively).

The morphological characteristic of the vaginal smears of the healthy women, the patients with CV and the patients with VVC were observed under microscope, the 6 typical smears were captured and listed as follow (Figure 1). The *Lactobacillus* species appeared to be gram-positive rods (Figure 1.A.B.C.). At the same time, *Candida* spores appeared to be gram-positive oval cells that were smaller than erythrocytes; blastospores were spores with sprout; hyphae were extending germ tubes of blastospores (Figure 1.D.E.F). *T. vaginalis* was a single-celled flagellar parasite that was slightly larger than a white blood cell; it appeared to have an obpyriform or polymorphic shape; its nucleus was oval, located on the 1/3 fore-end of the organism; back to the nucleus there was an axostyle; 5 flagella arose near the cytostome but were usually not very clear by gram staining (Figure 1.E). Clue cells were epithelial cells covered with small gram-negative or gram-variable rods (indicative of *G. vaginalis*) (Figure 1.F). The observed characteristic of *Lactobacillus* species, *Candida* species, clue cells and *T. vaginalis* all highly agree with the descriptions provided in different studies.[2, 23-25]

Based on the quantity of *Lactobacillus*,[26] morphology of the epithelial cells, presence or absence of *Candida* species, *T. vaginalis* and clue cells, the morphological characteristics among patients with CV, healthy women and patients with VVC can be differentiated (Table 2).

Discussion

Gram staining of vaginal smears reveals the following morphological characteristics of CV: overgrowth of *Lactobacillus*, lysis of epithelial cells, the presence of fragments of epithelial cells and naked nuclei, absence of candidal spore, bla-

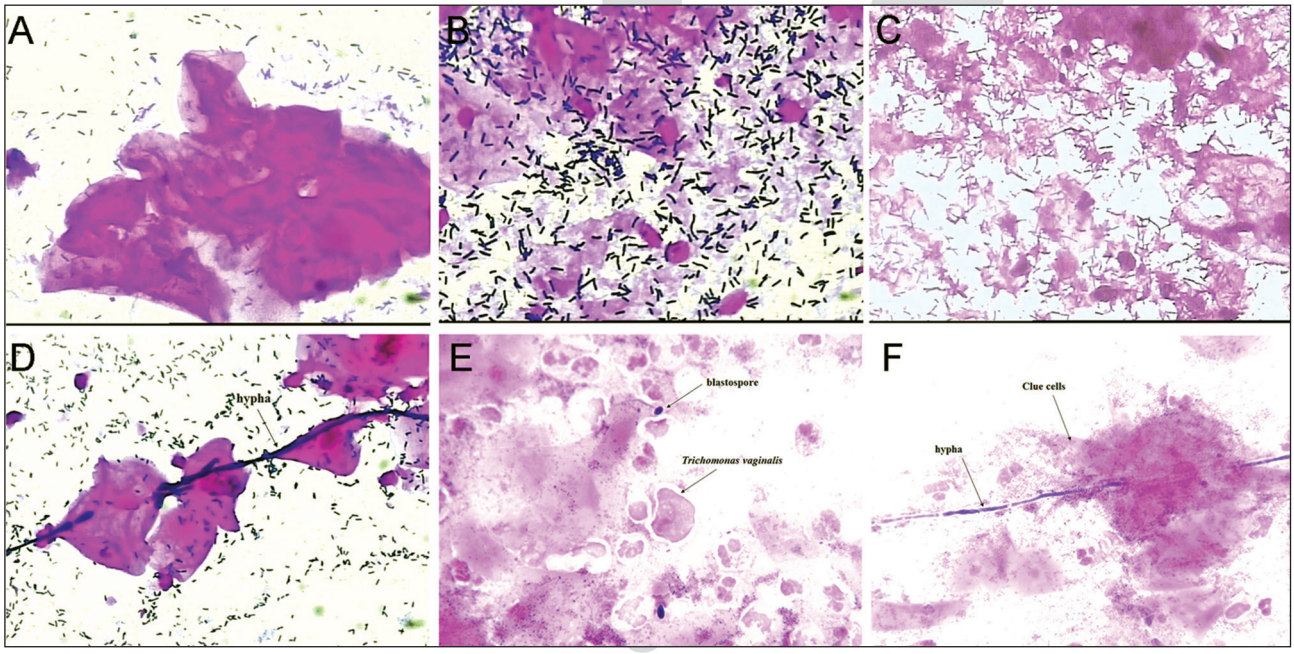

Figure 1. Microscopic observation of the vaginal smears of 6 typical subjects (gram staining)
A. Vaginal smear of the healthy woman: the presence of a large amount of Lactobacillus and normal epithelial cells (400×); B. Vaginal smear of the patient with mild cytolytic vaginalis (CV): presence of a large amount of Lactobacillus, naked nuclei, cytoplasmic fragmentations (1000×); C. Vaginal smear of the patient with severe CV: the presence of a large amount of Lactobacillus, naked nuclei, cytoplasmic fragmentations, lack of whole epithelial cells (1000×); D. Vaginal smear of the patient with a single infection of vulvovaginal candidiasis (VVC): the presence of a large amount of Lactobacillus, normal epithelial cells, candidal spore, blastospores and hypha (400×); E. Vaginal smear of the patient with combined VVC and trichomoniasis infection: presence of T. vaginalis, normal epithelial cells, candidal spore, blastospores and hypha (1000×); F. Vaginal smear of the patient with combined VVC and bacterial vaginosis infection: presence of Gardnerella vaginalis (clue cells), normal epithelial cells, candidal spore, blastospores and hyphae (1000×).

Table 2. Morphological identification of vaginal discharge of the patients with CV, the healthy women and the patients with VVC in this study

Subjects(n=108)	*Lactobacillus*	Fragments of epithelial cell	Whole epithelial cells	*Candida* species	Other pathogens
Healthy women (n=21)	10–999 per oif	Absent	All	Absent	Absent
Patients with mild CV (n=18)	≥1,000 per oif	≤50%	>50%	Absent	Absent
Patients with severe CV (n=15)	≥1,000 per oif	>50%	≤50%	Absent	Absent
Single infection of VVC (n=20)	10–999 per oif	Absent	All	Present	Absent
Combined VVC and Trichomoniasis infection (n=16)	0-9 per oif	Absent	All	Present	*T. vaginalis*
Combined VVC and bacterial vaginosis infection (n=18)	0-9 per oif	Absent	All	Present	Clue cells

CV, cytolytic vaginosis; VVC, vulvovaginal candidiasis; oif, oil immersion field

stospores or pseudohyphae, absence of other pathogens, and so on (Figure 1). Although CV and VVC share many symptoms such as pruritus and profuse vaginal discharge, significant differences in morphological characteristics are seen under the microscope. Furthermore, when a patient has a combination infection of VVC and another pathogen, the quantity of *Lactobacillus* decreases or disappears and clue cells or *T. vaginalis* is present instead (Table 2).

The following diagnostic criteria for CV have been suggested by Cibley LJ et al[7]: high risk of suspicion; absence of *T. vaginalis*, *clue cells* or *Candida species* on a wet vaginal smear; increased numbers of *lactobacilli;* a paucity of white blood cells; evidence of cytolysis; discharge; and pH 3.5–4.5. Many of these diagnostic criteria were observed under the microscope, indicating the need for morphological observation by a microbiologist.

Compared to the above criteria, the current study has improved morphological observations in many ways. First, the absence of *T. vaginalis*, *clue cells* or *Candida species* was proved by using gram staining. Second, the differences in the morphological characteristics between patients with mild CV and patients with severe CV were successfully identified. Third, the quantitative difference between *Lactobacillus* overgrowth and normal quantity was identified. And fourth, the morphological identification of vaginal discharge in a healthy woman, patients with CV and patients with VVC was performed by our research team.

Our study has some limitations. First, although the 6 typical smears were observed under microscopes which were made by the same company, but they were photographed by different image acquisition systems, which resulted in different image effects. Second, the diagnosis of VVC should be based on direct microscopic examination of a wet mount preparation or a vaginal culture.[27, 28] However, for the standardization of the observational method, the 3 cases of VVC were diagnosed only by the abnormal vaginal discharge and examined morphologically using gram staining. Finally, there are no specific criteria listed for the number of lactobacilli, epithelial cells, clue cells or organisms used to classify the entities studied, which will be improved in the further study.

Conclusion

Using the morphological identification process in this study, including the quantity of lactobacilli, the morphology of epithelial cells and the absence or presence of *Candida* species and other pathogens, CV and VVC can be differentiated and the misdiagnosis of CV as VVC can be avoided.

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Effect of topical application of Salmonella enterica lipopolysaccharide on excision wound healing in Balb/c mice

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Abstract

Background: Wound healing can be discontinued in immunocompromised states as a result of impairment of effector molecules. Endotoxines the major virulence factors of Gram-negative bacteria has been a surge of interest for their immunological, pathophysiological and pharmacological effects on eukaryotic cells. Lipopolysaccharide (LPS) is one of the major inducers of inflammatory mediators. The main purpose of this investigation is to evaluate the effect of topical administration of *Salmonella enterica* LPS on excision wound healing in Balb/c mice.

Material and Methods: The assessment of cyclooxygenase-2 (COX-2), hydrogen peroxide (H_2O_2) and nitric oxide (NO) expression were carried out for detection of their possible role in wound healing process. Animals were devided in to four groups. Tissues were harvested at 1, 2, 3 and 7 days for biochemical and histopathological evaluations of wounded skin tissues. The expression of selected mediators was determined using the related kits as per the manufacturer's protocols.

Results: The LPS treated wounds (100µg) was showed more infiltrations of inflammatory cells and slightly thicker epithelium layer than that of control wounds in pathological investigations and higher expression of NO, H_2O_2 and COX-2 in biochemical assays ($P<0.001$). The results suggest that LPS has the potential to accelerate inflammatory phase of wound healing process by enhanced expressions of mediators.

Conclusion: These findings may be worthwhile in detection of new potential therapeutic agents for non-repairing wounds. Future studies covering effects of different bacterial strains with various doses will give us a better comprehension of the *in vivo* mechanism of action of bacterial components on wound healing process.

Key words: Excision wound model, lipopolysaccharide, inflammatory mediators.

Introduction

Inflammatory phase of wound healing process is subdivided in to vascular and cellular responses. During cellular response chemotactic factors released in hemostasis provide the recruitment of neutrophils and monocytes into the wound area (1). Among the chemical mediators of inflammation, arachidonic acid metabolites of prostaglandins, nitric oxide andoxygen-derived free radicals are known to be prominent factors with critical roles in wound healing (2,3). Prostaglandins and nitric oxide derived from nitric oxide synthase (NOS) and cyclooxygenase are potent biologic substances for promotion of vasodilation, vascular permeability, neovascularization, matrix deposition and being beneficial to the healing process (4-7). Synergistic activity of Prostaglandin E_2 and prostaglandin I_2 with other mediators can increase micro vascular permeability. Stimulation of fibroblast mitogenesis by $PGE_2$with interference of platelet derived growth factor (PDGF)-mediated signals can lead to cell proliferation in proliferative phase of wound healing.In early stages of wound healing; PGE_2 may be the stimulator of increased fibrosis (8). Despite the past studies, re-

active oxygen species (ROS) that were considered to have harmful effects on cells and tissues, recent studies suggest ROS (i.e. H_2O_2) function as redox signaling that stimulate cell adhesion, migration, vasculogenesis and wound healing (9,10).

One of the major inducers of inflammatory mediators is bacterial endotoxin called lipopolysaccharide. The relationship between nontoxic levels of LPS with wound repair in airway epithelial cells via activation of signaling cascade has been demonstrated (11). The scope of this report is the evaluation of effect of LPS on skin wound repair and development of therapeutic approaches will be discussed. Once LPS binds to LPS binding protein (LBP), recognition of LPS with Toll-like receptor 4 (TLR4) or TLR-2 can stimulate the intracellular signaling cascades that leads to activation of mitogen activated protein kinase (MAPK), nuclear factor-kB (NFkB), increased expression of COX-2 and ultimately enhanced production ofinflammatory mediators such asPGE_2, ROS and NO (6,12,13). During inflammatory response the relationship between NO and COX indicates the importance of NO in COX expression and also inhibitory affects of COX inhibitors on NOS activity (14).Recent researches implicated the essential role of NO for providing a cross-talk between nuclear transcription factor kappaB (NFkB) pathway and PGE_2in wound fibroblasts for regulation of collagen formation (15,16). The aim of this study is to evaluate the effect of topical administration of bacterial LPS on excision wound healing in Balb/c mice for detection of inflammatory mediators' role in wound healing process.

Materials and Methods

Materials

Lipopolysaccharide (LPS; *Salmonella enterica* L6511-100mg. 109k4087) were purchased from Sigma, Nitric Oxide assay kit (Biovision, Ca, USA), H_2O_2 assay kit (Cayman Company), COX-2 assay kit (Assay designs & Stressgen Inc. Michigan, USA), ketamine (Alfasan, Worden, Holand), xylazine (Alfasan, Worden, Holand), PBS, formalin 10%, gentamicin,

Experimental Animals

8-12 week old male Balb/c mice were obtained from animal laboratory of Pasteur Institute of Iran. For acclimatization of animals, they were maintained in a well-ventilated room, under standard condition of 19-21°C with 40-60% humidity and 12-h/12-h (light/dark) cycle for 1 week prior to experiment. The animals were housed one per cage and were fed with standard pellets and water*ad libitum*.The animal care and housing were carried out with no significant levels of contaminants.

Preparation of Excision wound model

Mice weighting between 20-30g were anaesthetized with a single injection of ketamine hydrochloride (i.p., 5mg/kg), xylizine (i.p., 5mg/kg) prior to and during the experiment. Subsequently, the hairs on the back of mice were shaved with electrical shaver and wiped with 70% ethanol. Four identical circular full-thickness wounds were generatedat the same distance on the dorsum of each mouse using a sterile biopsy punch (3mm). Animals were inflicted according to excision wound model as described by Morton and Malone. The skin tissues were dissected out carefully and the wounds were left open during the study. The wounding day was considered as day 0. Two of the Wounds at left side of the body were selected as Control wounds and the other two wounds at right side were treated with LPS. Animals were divided into four groups of five mice in each group, according to harvesting days for biochemical and histopathological analysis of skin tissues at wound sites: day 1, 2, 3 and 7. All the surgical procedures wereapproved by the animals committee of Pasteur Institute of Iran and carried out under sterile conditions for animals which were housed one per cage.

Topical application of LPS

To examine the inflammatory potential of lipopolysaccharide in vivo, LPS solution was applied topically directly to the wound bed, 24 hours after wounding day, once daily. As explain previously, two of four open wounds created on each Balb/c mice were treated with equal dose of LPS (100µg/ml). PBS was applied topically once in a day to the wounds selected as control group in the same manner.

Tissue lysate preparation

Tissue samples were homogenized with lysis buffer containing detergent phosphate buffer saline (PBS 0.05% Tween20) and complete protease

inhibitor (pro-block cocktails anti-protease, manufacturers Gouldbio Inc. USA). Homogenized samples were centrifuged 4000rpm/10min and the supernatants were collected for performing NO, COX-2 and H_2O_2 measurement.

LPS-induced skin inflammation and Determination of NO production

Nitric oxide levels in the above mentioned samples were determined using colorimetric assay kit (Nitric Oxide assay kit, Biovision, Ca, USA). The assay principle was Griess reaction, the assay sensitivity was 0.2 nM and the intera assay coefficient of variation was 3.4%.

LPS-induced skin inflammation and COX-2 level

The activity of COX-2 was measured by Enzyme Linked Immuno Sorbent assay kit (Assay design & Stressgen Inc. Michigan, USA) according to the recommendation of the manufacturer insert. The measured optical density in 450nm was proportional to the concentration of COX-2 in either standards or samples.

Hydrogen Peroxide Measurement

Hydrogen peroxide levels in the supernatants of homogenized tissue samples were determined using colorimetric assay kit (Hydrogen peroxide assay kit, Biovision, Ca, USA). The assay principle was peroxidase reaction, the assay sensitivity was 0.1nM and the intera assay coefficient of variation was 2.8%.

Histopathological evaluation of wounded tissues

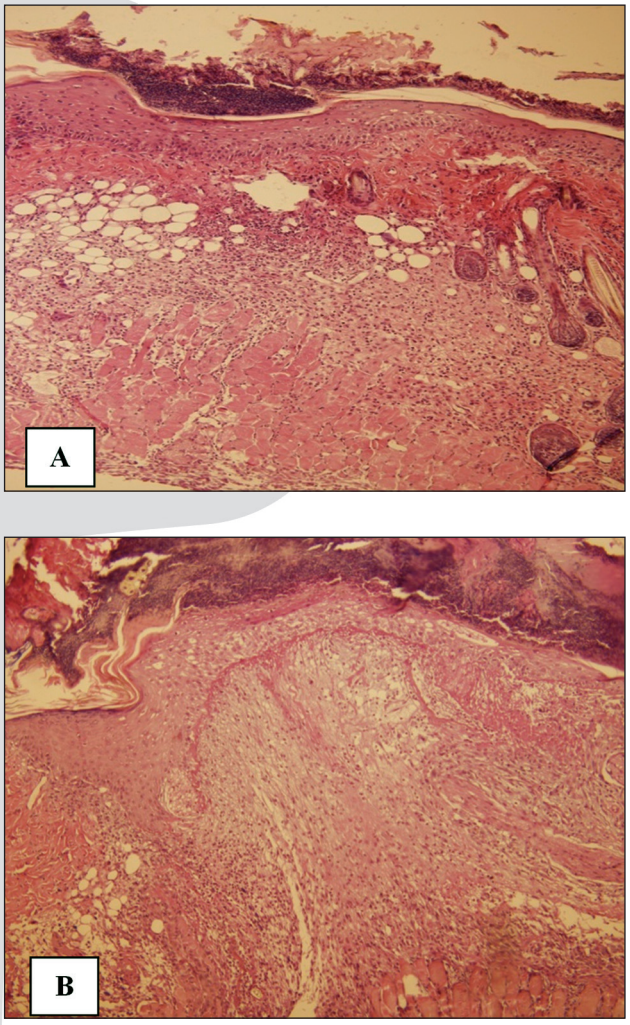
For histopathological analysis, 5 mice of each group at the days of 1, 2, 3 and 7 were sacrificed. The harvested skin wounds were included epidermis, dermis and subcutaneous connective tissue. Tissue samples were fixed in 10% formalin separately, processed by dehydrating through graded alcohol series, clearing in xylene and then embeding in paraffin wax. Thick slice were prepared (4-6μm) and stained with hematoxylin eosin (H&E).

Statistical analysis

Statistical analysis was performed by using SPSS (version 20) software. Levels of NO, COX-2 and H_2O_2 between control and test samples were analysed using ANOVA, followed by Student's *t* test. Values of $p<0.05$ were considered significant differences between groups. Data were expressed as means ± standard errors (SEM).

Results

There was no significant difference between treated and control group on day 1 and 2 after wounding day. Both of the groups had thin and incomplete epithelium layer with few vessels on these days.



Picture 1. Hematoxylin eosin (H&E) staining for histopathological compare in the character of wound healing between control and treated group on day 3. Treated group showed slightly thicker granulation tissue layer with plenty of inflammatory and repair cells (B). Control group in the left side showed thin granulation tissue with massive necrotic substances and less number of inflammatory cells (A).

Wounds of treated group compared to wounds of control group on day 3 displayed numerous inflammatory cells with fibroblasts and slightly thicker layer of epithelium. On day 7, wounds of both groups were healed.

LPS-induced skin inflammation and Determination of NO production

To investigate the effect of *Salmonella enterica* LPS on NO production in skin wound samples, Griees assay was performed. As shown in Figure 1. It seems that the effect of LPS on production of NO in wound site was increased after day 3 ($P<0.001$) and it continued to day 7. The highest NO level for the control group was on day 2 and it decreased after day 3.

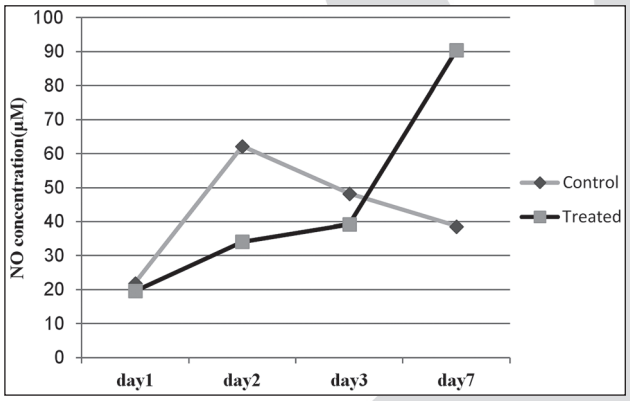


Figure 1. Effect of LPS (100µg) on NO production in skin wound samples at 1, 2, 3 and 7 days after wounding day

LPS-induced skin inflammation and COX-2 level

The effect of LPS on COX-2 level in wounded skin samples was shown in Figure 2. Results indicated that peak level of COX-2 was observed on day 3 and after that COX-2 was decreased.

Hydrogen Peroxide Measurement

The results obtained from H_2O_2 measurement indicated (Figure 3.) that the highest level for treated group was on day 2 and then decreased ($P<0.001$). But in the control group its level increased at early stage, then decreased and remained constant until day 3. It seems that the amount of H_2O_2 in the early phase was increased but in the late phase it was decreased after LPS treatment.

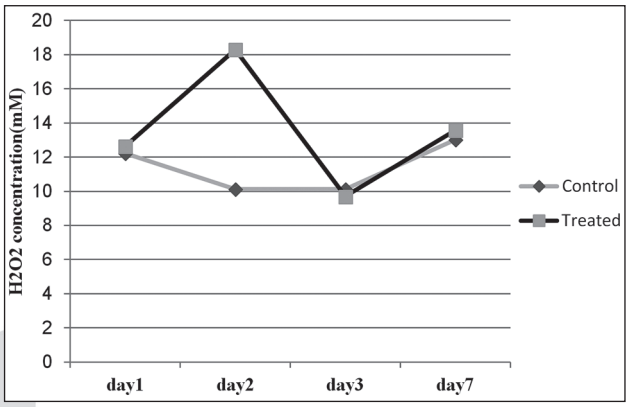


Figure 2. Effect of LPS (100µg) on COX-2 level in skin wound samples at 1, 2, 3 and 7 days after wounding day

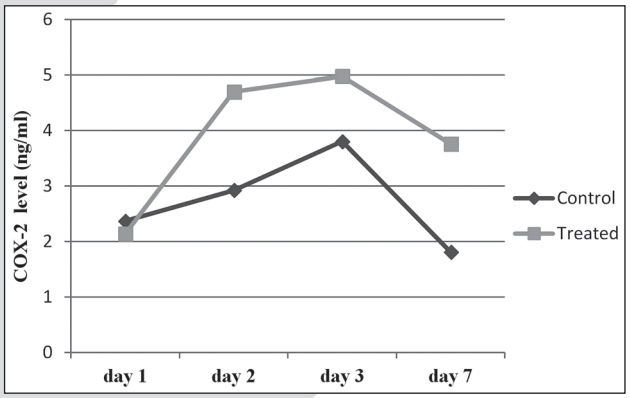


Figure 3. Effect of LPS (100µg) on H_2O_2 level in skin wound samples at 1, 2, 3 and 7 days after wounding day

Discussion

Diabetic and other immunocompromised Patients suffering chronic wounds impose economic and societal burden worldwide. These health care risks are the consequence of impaired production of effector molecules such as nitric oxide and decreased number of immune cells in immunocompromised states that make a force in detection of novel potential therapeutic agents for non-repairing wounds (21-23). The regulation of inflammatory phase is an inevitable factor of wound healing process. In order to quick wound healing without infections, rapid inflammatory response is required. Inflammatory response is associated with production of chemical mediators such as nitric oxide, cyclooxygenase-2 and hydrogen peroxide (1). Wound healing can be discontinued in immunocompromised states as a result of impairment of effector molecules (23). Decreased inflamma-

tory response can be the cause of reduced granulation tissue formation and reepithelization. Taken together, recent studies highlight the crucial role of inflammatory mediators in providing cutaneous wound healing (3).

In the present research, it was clearly demonstrated that topical treatment of Balb/c mouse wounded skin with LPS provoked nitric oxide and cyclooxygenase-2 induction. Nitric oxide and prostaglandins are associated in promotion of vasodilation, vascular permeability, neovascularization, matrix deposition and being beneficial to the healing process. It is well known that Bacterial lipopolysaccharides are the major inducers of inflammatory mediators (12). According to investigations that exert NO releasing compounds for wound healing studies, this research makes effort to illustrate NO enhancing potential of a bacterial endotoxin in wound model.

Recent similar studies that were performed by K. Blecherand et al. demonstrated that nanoparticle platform that can generate, store and deliver NO have profound wound healing properties (23). Jonathan L. Koff and et al. hypothesized that lower concentrations of LPS can accelerate wound repair in airway epithelial cells via activation of a novel signaling cascade (11). Diabetic wounds are charecterizad by low levels of nitric oxide in the wound bed. This low levels NO are due to the deficiency in nitric oxide synthase (NOS) enzyme (24).In 2012, N. Dashti and et al. indicates that the nitric oxide donor PAPA NONOate can increase the rate of collagen synthesis in wound that may have a therapeutic potential for diabetic impaired wound healing (25,26). NO is essential factor for angiogenesis. In 2003, A. Tas and et al. were evaluated the effects of sildenafil citrate (Viagra) in wounds. Sidenafil citrate is an inhibitor of phosphodiesterase type 5 (PDE5). Inhibitory effects of sidenafil citrate on PDE5 cause high levels of cyclic guanosine monophosphate (cGMP) which can increase the relaxation of smooth muscles and increase the effect of NO. According their result, the sildenafil citrate can enhance wound healing by stimulating angiogenesis (27).

In 2000, Marcelo N. Muscara, and et al. reported the effects of nitric oxide releasing derivative of naproxen (NO-NSAID) and a selective COX-2 inhibitor on collagen deposition in healing process. While the collagen deposition in wound site was decreased during naproxen administration, NO-NSAID had increased effect on collagen deposition. Both of the drugs were COX-2 inhibitors and suppressed prostaglandin synthesis in wound model, so the inhibitory effect cannot explain their reverse effects. According to their results, NO-NSAID was the enhancer of collagen deposition at wound site (28). In 2002, Kristyn S. Bohl Master, and et al. introduced nitric oxide releasing poly (vinil alcohol) hydrogel dressings which had potential of modulating chronic wounds (29).

In conclusion, this is the first report indicating in vivo evidence of LPS inflammatory potential as a topical wound healing agent. In our experimental condition, topical treatment of mice wounded skin with LPS result in more infiltrations of inflammatory cells and slightly thicker epithelium layer. Further studies of different doses of different bacterial strains will give a better understanding of the in vivo mechanisms of LPS on wound repair. These findings may be worthwhile in development of new methods for wound therapies.

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Fasting behavior of breastfeeding mothers in Ramadan

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Abstract

Aim: The aim of this study was to investigate fasting behavior of women who had a baby younger than six months old having exclusive breastfeeding.

Material and Methods: This study is descriptive and cross-sectional. The study sample included 101 mothers (age 26.9±4.9 years) who had babies younger than six months old and having exclusive breastfeeding. Data were collected with a form developed by the investigator.

Results: Almost all of the mothers (90.1%) noted that they had fasted in previous Ramadans and nearly 50% of the mothers also reported that they fasted in Ramadan in 2010 when they were breastfeeding. However, the rate of the mothers worrying that their breast milk production could be affected by fasting was quite high (91%). Age of babies, having the first child and beliefs that a woman can breastfeed while fasting were found to be significantly effective in mothers' fasting behavior ($p<0.05$).

Conclusions and Recommendations: Almost half of the women were fasting during their lactation period. Health staff does not carefully question fasting behavior of breastfeeding women likely to affect sufficient and balanced nutrition in the first six months of giving birth during which both mothers and their babies are supposed to be followed closely. It is important that health staff, especially those likely to encounter breastfeeding women in Ramadan should ask mothers whether they fast, should be able to evaluate variables which may play a role in fasting status of breastfeeding women and should answer questions asked by mothers about fasting and breastfeeding.

Key words: Breastfeeding, exclusive breastfeeding, Ramadan, fasting, behavior.

Introduction

Fasting in the month of Ramadan is one of the five pillars of Islam (1) and Ramadan is the holiest month of Islam (2). Fasting in Islam refers to not eating and drinking anything at all and avoiding sexual relationship from the sunrise to the sunset for 29 or 30 days in Ramadan, the ninth month of Lunar Calendar.

Since Ramadan is a Lunar month, it may sometimes be in winter and sometimes be in summer. Therefore, duration of fasting varies from 11 to 18 hours a day. In fact, fasting may last up to 18 hours in summer (1, 3).

According to Islamic principles, healthy individuals have to fast; however, patients, travelers, pregnant women, breastfeeding women, women experiencing their periods and individuals suffering from hunger and excessive thirst do not have to fast and they can postpone it (1, 4). In fact, if a pregnant or breastfeeding mother fears for herself or her child because of fasting, then she is allowed not to fast, but she has to make up the days that she does not fast.[1,5] However, some breastfeeding women are observed to fast (1, 3, 6, 7). It is explained by the fact that fasting is a social worship; i.e. all the people in the society start and finish fasting at the same time, and that women prefer to fast together with their families during Ramadan for spiritual reasons rather than postpone it and fast alone.

There have been few studies on fasting behavior of breastfeeding mothers and effects of fasting on lactation (5, 6). Bener et al. reported that fasting did not affect breast milk quality and content (8). Khodel et al. also noted that fasting did not have a negative effect on growth and development parameters in babies fed exclusively with breast milk (6, 9). Rakıcıoğlu et al. also reported findings consistent with the above studies; however, overall food consumption (except for some vitamins

A, E and C) decreased, though it did not have a significant effect on growth of babies (10).

We investigated fasting status in women with babies younger than six months old and having exclusive breastfeeding in this study.

Materials and Methods

This is a descriptive, cross-sectional study. Thirteen primary health care centers in the city of Aydın were considered as a cluster. One primary health care center was selected with simple random sampling and the area provided care by this primary health care center was considered as the study area. All the mothers who had babies younger than six months old and having exclusive breastfeeding were visited. Data were collected within one month after Ramadan (between 13 September and 11 November 2010) with a questionnaire created by the investigators. A hundred and twenty-three mothers registered in the primary health care center and fulfilling the study criteria were considered as the study population. Because the rest was unavailable, moved to another part of the city, died or rejected to participate in the study, data were collected only from 101 mothers. These mothers were considered as the study sample.

Study setting

One of the most strategic countries in terms of its geopolitical importance, Turkey is located on the crossroads of Asia, Europe and Africa, known as "Old World Lands". It is a single bridge not only between Eastern and Western civilizations but also between all religions. Aegean Region is the region of Turkey with the largest coast on the sea. It occupies land of about 79.000 km² and comprises 11% of all productive land of Turkey. The population of the region reminds a mosaic made up of many religions and cultures. Aydın, described by the famous historian Herodotus as "land underneath the most beautiful sky we know on the Earth", and where the study was conducted, is one of the cities in which nature and culture merge. It is a city where the rate of literate women is high[11] compared to the rate of literate women in Turkey[12] and almost all women received antenatal care and had their babies followed regularly[13]. The women living in the district where the study was conducted were at least primary school graduates and could be considered as having medium financial status.

The flow of the study

Ramadan was between 11 August and 9 September in 2010. The investigators planned this study when they found out that some breastfeeding women were fasting while they were attending nursing students' practicum for Public Health Nursing. During Ramadan, a questionnaire was created in the light of the literature and inclusion criteria were determined. After Ramadan, a list of the mothers with a baby younger than six months and registered in the randomly selected primary health care center was made and all the mothers in the list were visited until the end of September and data were collected from the women fulfilling the inclusion criteria and accepting to participate in the study. All the women participating in the study gave informed consent. Approval was obtained from Health Directorate of Aydin.

Data were analyzed with descriptive statistics, t test and Chi-square test.

Inclusion criteria:

- Residing in the district of the primary health care center where the study was conducted,
- Having a baby younger than six months old at the end of Ramadan (9 September 2010) (date of birth of babies was between 10 March and 10 August),
- Having a baby which receive exclusive breastfeeding at the beginning of Ramadan,
- Lack of a health problem in both mothers and babies,
- Not taking any medications likely to affect breastfeeding or breast milk (e.g. hormonal contraception),

Results

Several descriptive characteristics of the mothers and their babies are presented in Table 1. A hundred and one mothers participating in the study were aged between 17 and 40 years and 55.4% of the mothers were younger than 27 years (mean=26.9±4.9; median=27). Duration of education of the mothers ranged from 0 to 16 years and was eight years on average (mean=7.9±4.20; median=8, mode=28). Most of the mothers were a

housewife and had a nuclear family. The mothers had 1-4 babies, one third of the mothers had a single baby and 43% of the mothers had two babies.

At the end of Ramadan (9 September 2010), the babies of the mothers participating in the study were aged between 23 and 180 days (mean=98.4±48.3) and more than half of the mothers' babies (54.5%) were 90 days old or younger. All of the babies were being breastfed; however, about one of 10 mothers noted that they started to give food other than breast milk (water, herbal tea, formula etc.) in Ramadan. Almost all the mothers (90.1%) reported that they fasted in previous Ramadans; however, the rate of the mothers fasting in Ramadan at the time of the study was 50%. More than half of the mothers fasted for 15 days or more (mean=16.0±7.62) and about 40% of the mothers fasted for 21 days or more. We found out that one mother gave birth on the first day of Ramadan and started fasting when her baby was seven days old, continued fasting during Ramadan and fed her baby with breast milk. In fact, about one third of the mothers believed that breastfeeding mothers can fast and this is not harmful to their own and their babies' health. However, the rate of the women believing fasting may affect breast milk production was quite high (91%).

There was a significant difference between fasting status and age of babies, having the first baby, believing that breastfeeding women can fast and believing that fasting can affect breast milk production. Fasting mothers were older, had a shorter duration of education and more frequently started to feed their babies with food other than breast milk than those not fasting, though the difference was not significant (Table 2).

All the mothers noted that they took their babies to the primary health care center for vaccination, examination and follow-up. Only 12 mothers (11.9%) explained that health staff asked them whether they were fasting or not. In addition, only eight mothers (7.9%) noted that they asked health staff whether they could fast. Both the mothers asked by health staff whether they fasted and those who asked health staff whether they could fast (n=20) told that they were recommended not to fast since they were breastfeeding.

*Table 1. Mothers' Characteristics (N=101)**

Mean age	26.9 (±4.9)
Mean duration of education	7.9 (±4.2)
Mean age of babies	98.4 (±48.3)
Being a housewife	84 (%83.2)
Having a nuclear family	86 (%85.1)
Having the first baby	34 (%33.7)
Having a baby starting to be fed with food other than breast milk	11 (%10.9)
Fasting	43 (42.6)
Believing that breastfeeding women can fast	32 (%31.7)
Believing that fasting affects breast milk production	92 (%91.1)

**Values are N (%) except where otherwise noted.*

Table 2. Distribution of Mothers' Characteristics by their Fasting Status

	Fasting (n=43)	**Not fasting (n=58)**	
Age of mothers (mean±SD)	27.1±5.2	26.8±4.7	t=0.303, p=0.76
Duration of education (mean±SD)	7.3±4.3	8.4±4.1	t=1.25, p=0.21
Age of babies (mean±SD)	125.1±44.6	78.6±41.2	t=5.41, p=0.00
Having the first child	10 (%29.4)	24 (%70.6)	x^2=3.36, p=0.04
Rate of starting to give food other than breast milk	6 (%14)	5 (%8.6)	x^2=0.72, p=0.29
Having a nuclear family	36 (%83.7)	50 (%86.2)	x^2=0.12, p=0.47
Believing that breastfeeding women can fast	18 (%41.9)	51 (%87.9)	x^2=24.2, p=0.000
Believing that fasting affects breast milk production	35 (%38.0)	57 (%62.0)	Fisher's x^2=8.58, p=0.04

Discussion

Ramadan is a lunar month, which is sometimes in winter and sometimes in summer. Therefore, duration of fasting during a day varies. In this study, Ramadan was in summer (August-September). Fasting duration a day was about 16 hours and the average temperature in the city where the study was conducted was 35.5^{0}C in August. The highest temperature recorded in August was 43.8^{0}C. Ramadan on the days when the temperature is the highest and when the time of fasting a day is long causes even healthy individuals to have difficulty in fasting.

According to Islamic principles, breastfeeding women do not have to fast. However, in this study, 43% of the mothers having babies younger than six months were found to fast in Ramadan. Consistent with the results of this study, Ertem et al. reported that 51.8% of the breastfeeding women fasted and Gökdemirel et al. showed that the rate of fasting among breastfeeding women was 45.6% (5, 14). These findings indicated that religious ties and feelings are stronger than mother-baby bonding and motherhood feelings. The lower rate of fasting mothers in the current study might have been due to the higher level of education and younger ages of breastfeeding women and the time when and the place of the study.

Fasting is a social worship. Kridli noted that many pregnant and breastfeeding women preferred to fast in Ramadan since they found it more difficult to fast alone when they wanted to compensate for the fasting they postponed formerly.[1] In the present study, one mother started to fast when her baby was seven days old, which confirms Kridli's comments.

In this study, most of the breastfeeding women not fasting in Ramadan had their first baby and their babies were younger. Ertem et al. also emphasized that babies' age plays an important role in mothers' fasting behavior (5).

A higher rate of the fasting women believed that they could fast while breastfeeding, which is consistent with the results of the study by Ertem et al.[5] There are not any Islamic principles which forbid breastfeeding women from fasting. Islam requires that healthy individuals are responsible for fasting in Ramadan. However, breastfeeding women are left to make their own decisions to fast or postpone fasting. This tolerance might have caused the women to believe that they would not have difficulty in fasting while breastfeeding.

One of the most striking findings of the study was that 35 out of 43 women fasted although they believed that fasting affected breast milk production. There can be three explanations for this. First, the mothers might have had strong religious beliefs, which caused them to fast although they thought fasting were likely to affect breast milk production. Second, they might have found it difficult to compensate for fasting later alone. Finally, social pressure created by all other people fasting in Ramadan might have encouraged the women to fast.

In the present study, the babies of the mothers included in the study were having exclusive breastfeeding at the beginning of Ramadan. A higher rate of the fasting women started to feed their babies with food other than breast milk such as water, tea and formula in Ramadan than that of the women not fasting without a significant difference. Gokdemirel et al. and Ertem et al., however, reported a significantly higher rate of the fasting women who started to give additional food to their babies (5, 14). This conflicting finding might have been due to the very low rate of the women who started to give additional food in the current study.

To the best of our knowledge, there have been few studies on fasting behavior of breastfeeding women and they have been performed to reveal effects of fasting on breast milk production and growth and development of babies. It has been noted that a mother with medium malnutrition can produce normal, good quality breast milk, and that a mother with severe malnutrition can produce sufficient breast milk if her baby frequently sucks, though the breast milk contains less fat and vitamin (WHO/UNİCEF). In addition, although fasting mothers' breast milk has lower concentrations of some vitamins and minerals, this does not affect growth and development of babies (6, 9, 10). However, Rashid found results of the studies showing no effect of fasting on babies fascinating, he noted that they could not be considered as strong evidence (2). Rakicioglu reported decreased intake of nutrients except for A, E and C vitamins among mothers fasting while breastfeeding and noted that especially intake of food containing calcium decreased (10). Therefore, to

what extent fasting during lactation, especially in the first three months of birth during which babies grow fast affects maternal health in the short term and the long terms can be an important subject of research.

Conclusion

To conclude, as in all religions, there are religious rituals in Islam likely to have positive or negative effects on specific groups of people. One of these groups which can be affected easily is the group of women breastfeeding and just giving birth. Therefore, health professionals should ask breastfeeding women whether they fast in Ramadan. If breastfeeding women want to fast, they should be encouraged to consume sufficient amounts of all nutrients and discouraged to give additional food to their babies. If breastfeeding women are indecisive about fasting, they should be offered religious information and results of relevant studies and convinced that they can postpone fasting and that postponement of fasting is the best option for both themselves and their babies.

Key messages:

- Some breastfeeding women prefer to fast in Ramadan. They should be convinced that they can postpone fasting based on Islamic principles.
- Mothers fasting in Ramadan should be discouraged from starting to give additional food to their babies.
- Women fasting during lactation should be provided training for nutrition and encouraged to eat sufficient amounts of all nutrients.
- Health staff should be offered training in order that they can ask breastfeeding women about their fasting behavior and provide them with appropriate recommendations.

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The genetic polymorphism of diabetic nephropathy

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Abstract

Introduction: The Diabetic Nephropathy (DN) affects approximately 40% of patients diagnosed with DM is associated with increased mortality from cardiovascular phenomena and is considered the main cause of Chronic Renal Failure (CRF) in patients dialectics.

Methods: Searches were performed on Medline, SciELO, Lilacs and Cochrane databases using the crossing between the key-words: "genetic polymorphism" and "diabetic nephropathy".

Results: The selected studies indicated that diabetic nephropathy is the leading cause of chronic renal failure, which significantly reduces the life expectancy of diabetics. Currently, some factors may have connection with DN. They are: genetic predisposition based on family history, hypertension, and cardiovascular events, quality of glycemic control and lipid levels and blood pressure and smoking.

Conclusion: Studies constants are essential to add new elements in the literature for the definition (s) of factor (s) gene (s) specific (s) of diabetic nephropathy.

Key words: Diabetic Nephropathies, Polymorphism, Genetic.

Introduction

The Diabetic Nephropathy (DN) affects approximately 40% of patients diagnosed with diabetes mellitus (DM) and it is associated with increased mortality from cardiovascular disorders. It is considered the main cause of Chronic Renal Failure (CRF) [1]. A review [2] cited it as responsible for 45% of cases of CRF on dialysis in the United States, 36% in Germany, 32% in Japan 15-25% and in several other European countries, apart from Australia. In Brazil and Latin America in general [3], about 15% of dialysis patients are diabetics.

Most diabetic patients with CRF are evolving with type 2 DM (DM-2) [4]. This finding takes on greater importance because of the prevalence of DM-2 is increasing in the world, according to the World Health Organization. There is also a higher incidence due to the global epidemic of obesity in developed and developing countries. More than 50% of global glomeruloesclerosis have clinical or pathological evidence that attribute diabetic sclerosis nephropathy [4].

The importance of ND is not only due to its high prevalence, but also because DM patients with proteinuria present a relative risk of premature death than not diabetic population [5]. In contrast, no diabetic nephropathy patients have twice the rate observed in non diabetics [5-6].

According to a sense of the Brazilian Society of Nephrology, the prevalence of CKD is 390 dialysis patients per million population, with about 73,600 in Brazil in dialysis patients. Although no systematic epidemiological studies in Brazil, it is estimated that DM is responsible for approximately 18% of dialysis patients in Brazil. There was an increase in the prevalence of CRF on dialysis secondary to ND in the last two decades. Studies [7-8] also estimate that 20- 45% of diabetic patients develop ND 10 to 15 years.

The World Health Organization estimates that up to 2025 there will be an increase in the prevalence of DM in the world, reaching a number of 300 million people. In developing countries, like Brazil, the increase is 170%. This increase, coupled with the increase in life expectancy of the general population can generate a significantly greater amount of patients with ND and dialysis [9].

Metabolic syndrome brought about by DM alone is enough to generate the glomerular lesions observed in ND. It is worth noting that these injuries can be prevented or have their intensity greatly diminished by the mere taking of blood glucose levels close to normal, always taking into

account the duration and intensity of treatment with insulin [10]. The interesting thing is that such renal lesions are reversed when an affected kidney is transplanted into an animal not diabetic [11].

In view of the above consideration, in this study we described the genetic polymorphism of diabetic nephropathy through a literature review.

Method

Search strategy and selection

The revisions were made between September 2012 and January 2013. The Medline (via PubMed), Lilacs, Scielo and Cochrane databases were searched using the following subject keywords: "genetic polymorphism" and "diabetic nephropathy". These words were defined by the Medical Subject Headings (MeSH).

The studies were selected by a reviewer and supervised by a senior reviewer. Based on the titles and abstracts, we excluded manuscripts not clearly related to the subject of the review. Thereafter, all the selected titles and abstracts were submitted to a final evaluation, which considered the inclusion criteria, and its reference lists independently checked to identify studies of possible relevance that were not found in the electronic search.

We excluded studies that presented no abstract or full text in English between 2000 and 2012 and literature reviews. As inclusion criteria we considered clinical trials and basic studies that investigated the effects of auditory stimulation on the ANS.

Results

The electronic search yielded a total of 898 references. Among these references the first elimination resulted in the exclusion of 837 titles and abstracts, which were not clearly related to the subject of review. The titles of the remaining 61 abstracts were submitted to a final evaluation that took into account the inclusion criteria. After the final exclusion of studies that did not investigate the SSCS, we finished the review with five references. The investigation of the reference lists confirmed the absence of relevant documents. Summaries of the main studies analyzed were selected.

Discussion

Diabetic nephropathy is the leading cause of chronic renal failure, especially in developed and developing countries, which significantly reduces the life expectancy of diabetics [12]. The DN gains importance as both the incidence of DM-2 is increasing and the beginning of its development has been increasingly early [12-13].

The prevalence of DM-2 is ten times greater than the DM-1, then it is observed by a greater prevalence of NA to CRF dialysis secondary to DM-2, both in foreign literature as national [14-15]. It is known that diabetic dialysis patients have lower survival rates than non-diabetics, which stems from an atherogenic vascular involvement that develops in a more accelerated [16].

A prospective cohort study followed 1853 dialysis patients with end-stage renal disease, 281 of these had ND as the main cause of dialysis, 107 had diabetes as a comorbid condition and 1465 did not have diabetes. The Dutch study of 2011 patients followed until they undergo the transplant or die, and the last outcome was common to 787 patients. Compared with nondiabetic patients, the risk of death was higher in both groups of patients, both those with ND as the main cause of dialysis (HR: 1.9) as those with diabetes as a comorbid condition (HR: 1.7) [17].

Coronel et al. in Madrid in Spain followed patients for at least 2 months of peritoneal dialysis for 25 years [18]. The study involved 118 diabetic patients of whom 66 had DM1 and 52 had DM2, and 117 patients who did not have diabetes. Number of hospitalizations and the same time were higher in diabetic patients than non-diabetic patients and there was no difference between patients with DM1 and DM2. Admissions due to cardiovascular problems were more common in patients with DM2. Mortality was 22% among those who did not have diabetes and 48% among carriers of the disease and in the last group mortality was higher in patients with T2DM.

In summary, many polymorphisms have been the subject of study, without having found what would be a specific marker. A supposed future identification of one or more factors that can define a direct genetic susceptibility to diabetic nephropathy allows patients at higher risk to be targets of intensive therapies or preventive interventions.

Unlike knowledge about genetic factors involved in ND, currently some nongenetic factors are recognized and clearly defined as its connection with the ND. It may be included genetic predisposition based on family history (patients in first degree) of hypertension and cardiovascular events, quality of glycemic control and lipid and blood pressure levels, besides smoking [19-20].

Among these factors it is included hypertension, its prevalence is approximately two times higher in diabetic patients compared to the normal population [21]. This relationship is conditioned to the fact that hypertension has a predictive factor for developing diabetes, but there is also a close relationship between hypertension and the development of ND. The prevalence of hypertension increases in ND concurrently with the severity and progress of IRC [22].

Katayama et al. [23] in a prospective 8-year follow-up also established a relationship between ND and rates of microalbuminuria. The Japanese study involving 1558 patients with DM2, showed progression of nephropathy in 74 patients. The rate of patients who progressed to ND was higher in patients with low microalbuminuria when compared to patients with microalbuminuria normal.

Faced with uncertainty about the genetic polymorphism of Diabetic Nephropathy, both in patients with DM-1 and DM-2, the correction of non-genetic factors associated with disease becomes key strategy for reduce prevalence and delayed clinical progression of the disease already established. Speak up on glycemic control, lipid and blood pressure, essentially.

Some studies have been done only in a particular country, which could characterize something typical of a certain ethnic group or population, restricted to their own cultures, having only a potentially regional applicability. Increasingly looking at isolated studies, what would be the main genetic factor of relevance, and several polymorphisms have been cited as involved in the pathophysiology of ND. Addressing genes in other populations previously studied in some research centers might create greater truthfulness of direct relationship, or lack thereof, of the genetic polymorphism in question with ND, excluding the obstacle of dealing with a polymorphism restricted to regional or an ethnicity or race.

Showing the influence of regional ethnic customs of a given population, cultural habits and even local genetic tendencies, compared to two recent studies in the literature regarding the main cause of kidney failure. The first, in 2012, conducted in the city of Jenin, Palestine, involved 84 patients with CRF, who were followed for 1 year. The study pointed to the DM as a cause of renal failure in 33.32% of patients [24]. The second study, in 2011, developed in Siri Lanka, followed 200 patients with chronic kidney disease for 3 months. Diabetes is the leading cause of CKD in 88.44% of patients [25].

Two distinct populations of one of the Middle East and other Asian continent, in presenting such a discrepancy in the pathogenesis of CKD, indicate the need to take into account the influence of regional factors in local studies. Also, there is the need for global multicenter studies evaluating genetic and non genetic factors common to different populations, to determine the precise pathogenesis of ND [26, 27].

Conclusion

The study of genes provides a wide researchers, the raw material, which is increasingly encouraged to go to search for new factors and new discoveries. Polymorphism of Diabetic Nephropathy not show different about this wide variety of new discoveries and constant studies are essential to the definition (s) of factor (s) gene (s) specific (s) of Diabetic Nephropathy.

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Acute effect of aerobic and anaerobic exercise on lipid levels

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Abstract

Purpose: The purpose of this study was to compare the acute effect of aerobic and anaerobic exercise on blood lipid levels. Another aim was to compare the blood lipid between pre-exercise, 1 minute and 60 minute after exercise levels.

Methods: 25 male students attending at the school of physical education and sport participated the study (age 22.55±2.37 years; sports age 8.51±3.34 years). The test of aerobic exercise (shuttle run) and the test of anaerobic (running anaerobic sprint) were applied to the subjects with one week intervals. Blood sample was drawn pre-exercise (resting), 1min after and 60 min after both aerobic and anaerobic exercise. Triglycerides, Total Cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) levels were examined.

Results: Triglyceride, total cholesterol, HDL-C and LDL-C levels that measured at resting, 1 min after and 60 minutes after exercise were not found different between aerobic and anaerobic exercise. it was identified that the levels of Triglyceride, total cholesterol, HDL-C and LDL-C measured at 1 minute after aerobic and anaerobic exercise were higher that the levels of pre- exercise levels. But 60 minute after exercise, the levels of blood have returned the level of pre-exercise.

Conclusion: No difference in TG, TC, HDL-C and LDL-C were observed among aerobic and anaerobic exercise trials. All blood levels transiently increased immediately after both shuttle run aerobic and RAST anaerobic exercise but returned to baseline levels by 1h post exercise. No significant differences were found for the blood variables across time or between treatments.

Key Words: Aerobic exercise, anaerobic exercise, acute effect, lipids.

Introduction

For a long time blood lipids of physical activity and their effects on cholesterol levels have been researched by various researches. In the most studies, it has been noticed that regular exercise affects levels of lipid and lipoprotein positively and it is salutary for the heart. It has been identified that regular exercise reduces total blood cholesterol, serum triglycerides and LDL-C and increases high-density lipoprotein cholesterol HDL-C (1, 2).

There are a lot of studies that have examined the acute effect of exercise on lipids (3). But it has been noticed that there are conflicting results dealing with the effect of acute exercise on the levels of lipid and cholesterol. Whereas some researchers have put forward that even an acute physical exercise has changed their parameters (4, 5) others have declared that this change will emerge at the end of long-term regular exercise (6, 7).

The results vary, but key factors affecting the results are the physical fitness of the subjects, the subjects' pre-exercise lipid levels, and the intensity and duration of the exercise session. Many of the studies used prolonged endurance exercise to determine whether any acute changes occurred. (3). In our study lipid levels were compared after aerobic and anaerobic exercise in trained individuals. Also in our study, the duration of exercise with both aerobic and anaerobic qualifications is more short-lived than the study researched previously. The aim of this study was to identify the acute effect of two different dose of exercise on lipid profiles. Another aim was also to research whether or not there are differences of blood levels between pre-exercise and 1minute and 60 minute after exercise.

Method

25 male students attending at the school of physical education and sport participated the study

(age 22.55±2.37 years; sports age 9.51±3.34 years). Height (cm) and body weight (kg) were measured and recorded. The mean and standard deviation of height and body weight are 181.11 ± 6.35 cm, 78.23 ± 9.06 kg respectively. The test of aerobic exercise (shuttle run) and the test of anaerobic (running anaerobic sprint) applied to the subjects with one week intervals. All subjects have applied to both exercise protocols but which test will be used firstly has been identified randomly. While 12 ones of the subjects were firstly practicing aerobic, other 13 ones of them practiced anaerobic. The participants warned that they do not practice middle and high-density exercise, drink alcohol and cordial.

Blood Measurements

Blood sample was drawn by doctor and specialist nurse before exercise (resting), 1min after and 60 min after both aerobic and anaerobic exercise. The samples of blood collected were transferred into tubes not including anticoagulant. After clotting it was shared into serums by being centrifuged at 3000rpm at 10 minutes, and divided serums were hid at -80^{0}C until the day of study. The levels of blood have been determined with ELISA by being used commercial kits in research laboratory of biochemistry department of Ondokuz Mayis University Medicine Faculty. Triglyceride, Total Cholesterol, HDL-C, LDL-C levels were examined.

20 m Shuttle Run Test

20m shuttle run test was performed for the provision of aerobic fatigue. New Test-Power Timer 1.9.5. (Newtest, Oulu, Finland) was used. Two photocells have been put into placed to start and end point of 20m running distance of the subjects. This test involves continuous running between two lines 20m apart in time to recorded beeps. Subjects stand behind one of the lines facing the second line, and begin running when instructed by the Power Timer. The subject continues running between the two lines, turning when signaled by the recorded beeps. Running speed has increased as 0.5km/minutes at each minute and warning has been given by voice of 3 bip. The test is stopped if the subject fails to reach the line (within 2 meters) for two consecutive ends.

Running Anaerobic Sprint Test (RAST)

Each subject warmed up for a period of five minutes which was followed by a five-minute passive recovery. Newtest-Powertimer 1.9.5. (Newtest, Oulu, Finland) was used for this test. This test has been included for the provision of anaerobic fatigue. The athlete completes six 35-metre runs at maximum pace with 10 seconds allowed between each sprint for turnaround.

Statistical analysis

SPSS 19 software packet was used for the statistical analysis of the data. All values are expressed as means ± standard deviation. Paired t test was used to search the differences of aerobic and anaerobic exercise trials. Repeated measures analysis of variance was used to compare the values of pre-exercise, 1 min after and 60 min after exercise.

Results

No significant differences were found between aerobic and anaerobic exercise in triglyceride levels that measured at resting, 1min after and 60 min after exercise ($p>0.05$).

Triglyceride levels that measured 1 min after aerobic exercise were significantly higher than that measured at 60min after exercise ($p<0.05$).

Triglyceride levels that measured 1 min after anaerobic exercise were significantly higher than that measured at resting and 60min after exercise ($p<0.01$).

Table 1. Triglyceride Levels for Aerobic and Anaerobic Exercise

	Exercise type	Resting (a)	p	1min after exercise (b)	p	60min after exercise (c)	p
Triglyceride	Aerobic	104.81±69.17	0.868	125.63±102.27	0.787	95.95±66.14	0.577
	Anaerobic	109.08± 51.32		126.64±61.40		105.28±50.77	
	Aerobic p	**b>c ***					
	Anaerobic p	**b>a,c****					

**p<0.05 and **p<0.01*

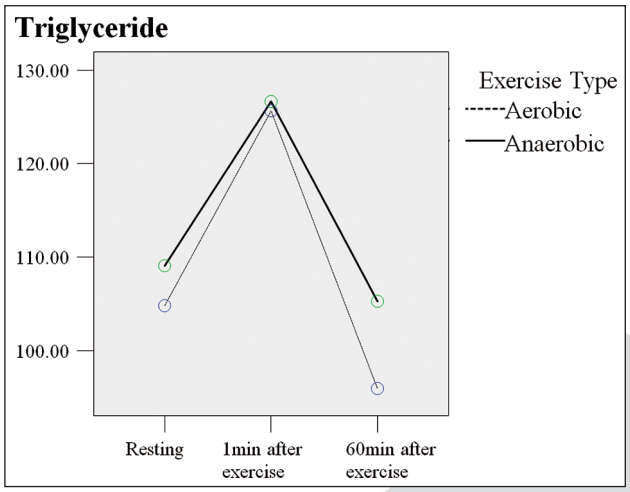

Figure 1. Triglyceride levels for aerobic and anaerobic exercise

No significant differences were found between aerobic and anaerobic exercise in total cholesterol levels that measured at resting, 1min after and 60 min after exercise (p>0.05).

Total Cholesterol levels that measured 1 min after aerobic exercise were significantly higher than that measured at resting and 60min after exercise (p<0.01).

Total Cholesterol levels that measured 1 min after anaerobic exercise were significantly higher than that measured at resting and 60min after exercise (p<0.01). Cholesterol levels were significantly lower after anaerobic exercise than resting levels (p<0.01).

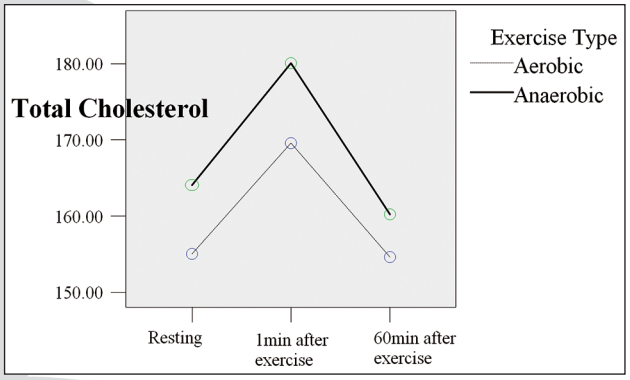

Figure 2. Total cholesterol levels for aerobic and anaerobic exercise

Table 2. Total cholesterol levels for aerobic and anaerobic exercise

	Exercise type	Resting (a)	p	1min after exercise (b)	p	60min after exercise (c)	p
Total Cholesterol	Aerobic	155.04±36.79	0.233	169.59±42.40	0.201	154.63±36.21	0.938
	Anaerobic	164.08±34.70		180.08±39.55		160.24±33.87	
	Aerobic p	**b>a,c ****					
	Anaerobic p	**a>c** b>a,c****					

***p<0.01*

Table 3. HDL-C Levels for aerobic and anaerobic exercise

	Exercise type	Resting (a)	p	1min after exercise (b)	p	60min after exercise (c)	p
HDL-C	Aerobic	47.35±10.50	0.696	51.02±11.39	0.607	47.07±10.37	0.989
	Anaerobic	47.16±6.76		51.24±7.43		46.58±6.25	
	Aerobic p	**b>a,c ****					
	Anaerobic p	**b>a,c****					

***p<0.01*

Table 4. LDL-C Levels for Aerobic and Anaerobic Exercise

	Exercise type	n	Resting (a)	p	1min after exercise (b)	p	60min after exercise (c)	p
LDL-C	Aerobic	25	88.78±32.65	0.465	96.56±36.90	0.487	90.60±32.54	0.757
	Anaerobic	25	94.90±26.65		103.49±29.56		93.16±26.71	
	Aerobic p	25	**b>a ***					
	Anaerobic p	25	**b>a,c****					

**p<0.05 and **p<0.01*

No significant differences were found between aerobic and anaerobic exercise in HDL-C levels that measured at resting, 1min after and 60 min after exercise (p>0.05)

HDL-C levels that measured 1 min after aerobic exercise were significantly higher than that measured at resting and 60min after exercise (p<0.01).

HDL-C levels that measured 1 min after anaerobic exercise were significantly higher than that measured at resting and 60min after exercise (p<0.01).

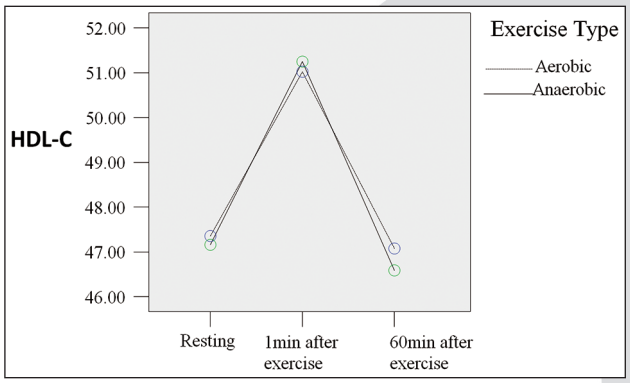

Figure 3. HDL-C levels for aerobic and anaerobic exercise

No significant differences were found between aerobic and anaerobic exercise in LDL-C levels that measured at resting, 1min after and 60 min after exercise (p>0.05)

LDL-C levels that measured 1 min after aerobic exercise were significantly higher than that measured at resting levels (p<0.05).

LDL-C levels that measured 1 min after anaerobic exercise were significantly higher than that measured at resting and 60min after exercise (p<0.01).

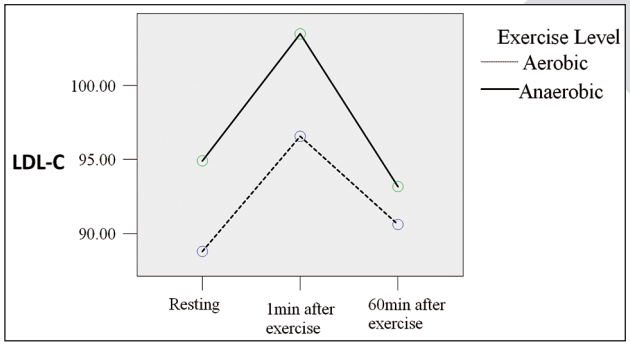

Figure 4. LDL-C levels for aerobic and anaerobic exercise

Discussion

In this study values of the blood lipids after shuttle run aerobic exercise and RAST were compared. The test of aerobic shuttle run took 390.56±84.56 seconds and RAST took shorter. Triglyceride, total cholesterol, HDL-C and LDL-C levels that measured at resting, 1 min after and 60 minutes after exercise were not found different between aerobic and anaerobic exercise.

Also blood lipid values were compared between pre-exercise, 1 minute after and 60 minute after exercise. In the present study it was identified that the levels of Triglyceride, total cholesterol, HDL-C and LDL-C measured at 1 minute after aerobic and anaerobic exercise were higher that the levels of pre-exercise levels. But 60 minute after exercise, the levels of blood have returned the level of pre-exercise.

In the study of Angelopoulos and Robertson (1993), seven sedentary men were studied during and after treadmill exercise at 65% VO2 max to determine the effect of a typical acute exercise bout on serum triglycerides. A venous blood sample was drawn immediately prior to a 30 minute treadmill exercise session, 5 min, 24 and 48 h after exercise. There were no significant differences in triglycerides and total cholesterol at 5 min, 24 and 48 h after exercise than pre-exercise. Only total HDL-C was higher at 5 min than pre-exercise (8). Also in our study HDL-C levels that measured 1 min after aerobic and anaerobic exercise were significantly higher than that measured at resting. Berg et al. study (1983) results suggest that physical exercise induces an increased formation of HDL particles of lower density from HDL particles of higher density (4). Kantor et al. (1987) examined the effects of a single exercise session on lipid and lipoprotein concentrations. Subjects exercised on a bicycle ergometer at 80% of their maximal heart rate for one or two hours. Blood samples were drawn 24 hours before and at ten minutes and 24, 48, and 72 hours after exercise. They found higher HDL-C values after two hours exercise (9) Ferguson and his friends' study (1998) supports our study that HDL-C concentration was elevated immediately after exercise in the 1,500-kcal session (10) similarly in our study.

In the study of Davis et al. (1992), ten well-trained runners completed treadmill exercise on two

different occasions: a high-intensity session at 75% maximal oxygen consumption lasting 60 min and a low-intensity session at 50% maximal oxygen consumption lasting 90 min. TC, TG, LDL-C, and HDL-C levels did not differ immediately after exercise and 1 hour after exercise than pre-exercise for two different occasions (11). In our study, the blood levels were not found different between pre-exercise and 60 minutes after exercise as a similarity to Davis and his friends' study.

In the study of Goodyear et al (1990), blood samples were collected from 12 female runners 24 h before, 10 min after, and 1, 3, and 5 d after running a 42 km marathon. They found that triglyceride concentrations were elevated immediately following the marathon but returned to baseline levels by 24 h post-exercise (12). Also in our study similar to them triglyceride levels that measured 1 min after anaerobic exercise were significantly higher than that measured at resting.

In some previous studies unlike our study the fact that TC TG and LDL-C measured just after exercise and 1 hour after exercise have decreased or have not changed, has been noticed (5, 10, 13, 14, 15). It has been noticed that the level of total cholesterol just after exercise has been decreased (13). Again in the study of Bounds et al. (2000), after 70% VO_2 max exercise (lasts about 77min) TC and TG decreased immediately post exercise. But LDL-C and HDL-C did not differ than before exercise (14). The reason of their finding more different conclusions than our study can be that the durations of the performed exercises are different. In our study, aerobic exercise has taken average 390.56±84.56 seconds, and the duration of anaerobic exercise is shorter.

Cullinane et al. (1981) have identified that decline in LDL cholesterol 66 hours after submaximal bicycle crunches 30 minutes performed to sedentary. Triglyceride, total cholesterol, high density lipoprotein cholesterol measurements at 5 minutes and 1, 4, 18, 42, and 66 hours after exercise did not differ from preface concentrations (16).

In the study of Visich et al. (1996) endurance-trained men completed three- counterbalanced running trials at different energy expenditures. The exercise durations were 28 min, 41 min and 55 min. For each trial, blood samples were collected at 24 h pre-exercise, immediately post-exercise, 1 h post-exercise, 6 h post-exercise, and 24 h post-exercise. When the blood levels were examined immediately after exercise and 1 hour after exercise, TG values were found significantly lower than pre-exercise; TC, LDL-C and HDL-C values were not significantly different than pre-exercise (17). In these mentioned studies the duration of performed exercise is more longer than exercise duration in our study. The reason of the fact that they have found different conclusions can be the duration of the exercise.

In our study total cholesterol level 60 minute after aerobic exercise has returned to the resting level. But total cholesterol level 60 minute after anaerobic exercise was found lower than the level of resting. In this conclusion, acute anaerobic exercise has reduced total cholesterol according to resting condition. It has been understood that anaerobic exercise can reduce the total cholesterol level more than aerobic exercise.

In conclusion, no difference in TG, TC, HDL-C and LDL-C were observed among aerobic and anaerobic exercise trials. All blood levels transiently increased immediately after both shuttle run aerobic and RAST anaerobic exercise but returned to baseline levels by 1h post exercise. No significant differences were found for the blood variables across time or between treatments.

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Determination of University students' consumer behaviors regarding milk and milk products: The sample of Selcuk University

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Abstract

Milk, which contains essential nutritional elements for growth and development, is one of the significant foods to be consumed in infancy, adolescence, and elderly periods. After milk became available for consumption thanks to heat treatment, the nutritional and taste values of milk components are also changing. These changes are of great importance for consumers' preferences and milk consumption habits. Therefore, the study was planned to determine university students' consumer behaviors regarding milk and milk products. The study sample is composed of university students from various faculties at Selçuk University between March 2012 and May 2012. The questionnaire form includes questions about general information (age, gender, grade, parent's education level, the place the family lives and the place the children live) about the children, their milk and milk production consumption and the criteria they use when purchasing these products. SPSS 13.0 package software was used for data analysis. The number and % values for each question were shown in tables and necessary arithmetic means were taken. Data were assessed with chi-square (χ^2) analysis by considering each variable. Simple random sampling method was used in the determination of the students and 311 volunteered students participated in the study. It was determined that 85.5% of the students consumed plain milk and 56.0% consumed whole fat and fat milk, 60.7% purchased processed packaged milk, 61.4% preferred milk products instead of milk. When purchasing milk and milk products, students firstly paid attention to expire date (80.4%), later the price (71.4%) and the brand (62.4%).

Key words: Milk, university students, consumer, milk products.

Introduction

University life is an important period in the individual lives of people as the food preference responsibilities of youth increase and for laying the foundations for a health life. A healthy and regular diet of university students is important for contracting or prevention of some diseases in the advanced ages. Balanced and regular diet is dependent on the intake of staple food products. While student who live with their families during their university education do not experience a great change in their food consumption habits, the fresh fruit, raw and cooked vegetable and sea food products consumption of the students who attend a university away from their families decreased and their consumption of fast food, alcohol and sugar increased [1].

However, milk and milk products which are the most important food of animal origin have a significant place for the nutrition of society [2]. Milk which is a perfect food has been one of the fundamental elements of nutrition for centuries all over the world. Thanks to vitamin A it has, milk, which is known to prevent osteoporosis, dental decay, stomachache and intestinal cancer contributes to the cleaning of the lungs and thus to the prevention of chronic bronchitis and even the lung cancer. Besides, calcium in milk inactivates the harmful substances that harm the cells in the walls of veins and protects high tension. Its other benefits are: providing energy to the brain, being effective against microbial infections, regulating the digestion system [3]. Milk, which has a significant place in the whole life of human beings includes protein, fat, lactose, vitamin and mineral at adequate levels necessary for an adequate and balanced diet. Besides its high nutritive value, milk is also a staple food which plays a significant role in regulating body functions and formation of bones and teeth

[4]. While in the Food Pyramid the United States Department of Agriculture (USDA) recommends that a healthy adult should consume 2-3 portions (200-400 ml) of milk and milk products, in the Nutrition Guide published by the National Milk and Milk Products Council, 2 to 4 (400-800 ml) portions, in the Nutrition Guide Peculiar to Turkey it is recommended that adult individuals should consume 2 portions of milk or milk products (one portion equals to 200 ml of milk), for children, adolescents, pregnant women and breastfeeding mothers, post-menopausal women are to consume 3 to 4 (600-800 ml) portions of milk or milk products [5]. According to recent data milk and milk product consumption per person was 173 kg in 2009, which is a rather low value [6].

Therefore, this study was carried out to determine university students' consumption of milk and milk products which are essential for healthy development of youth and the criteria they use when they purchase these products. The sub-aim of the study is to make valid recommendations to milk and milk products enterprises and marketers.

Material and Method

The sample of the study

The sample of this study which was carried out determine university students' milk and fermented milk product consumption habits is composed of undergraduate students attending faculties on Alaaddin Keykubat Campus of Selçuk University, Konya. Simple random sampling method was used to determine the students and 311 students from 14 different faculties and 2 vocational high schools participated in the study.

Data collection tools

Study data was collected via a questionnaire form administrated between April 2012 and May 2012. Before the questionnaire form was formed, relevant literature about the topic (dissertations, articles, paper, scientific research and the like) was reviewed and the questionnaire form was prepared using relevant resources and previous studies [7;8]. Questionnaire form included various questions about students and their families, milk consumption conditions and the criteria they use when purchasing milk and milk products. The study data was collected with the questionnaire form via face-to-face interview technique. The questionnaire forms were given to students at their faculties and canteens after necessary explanations were made and they were asked to fill in the form with their own will.

Data analysis

Data was submitted statistical analyses using Statistical Package for the Social Sciences (SPSS) 13.0 package program in Windows. Statistical assessment of the data obtained as a result of the study, tables showing mean, standart deviation (S) and percentage (%) values were prepared and chi-square (χ^2) significance test was used.

Demographic characteristics of the sample group

While 51.4% of the students attending this study which was carried out to determine university students' milk and fermented milk product consumption habits are female, 48.6% are male. Almost half of the students participating the study (41.2%) were aged between 20 and 21, 28.9% were aged between 22 and 23, 16.1% were 24 and over, which is followed by those aged between 18 and 19 with 13.8%. 29.6% of the students were junior, 25.7% were senior, 24.8% were sophomore and 19.9% were freshmen. More than half of the students (54.0%) resided in dormitories, which is followed by those who stayed with their families (27.0%) and those who shared a flat with other students (17.4%) and those who stayed with their relatives (1.6%). It was determined that fathers and mothers of the students were mostly primary school graduates (respectively %73.3, %44.7). Most of the students' families lived in the city center (87.5%) (Table 1).

Findings and Discussion

Under this heading, first of all relation between milk and milk products preference conditions of the students and demographic variables were. After that, the relation between criteria students observe when purchasing milk and milk products and demographic variables was examined.

Table 1. Socio-demographic characteristics of the students

	Male (n=151)		Female (n=160)		Total (n=311)	
	Number	**%**	**Number**	**%**	**Number**	**%**
Student's age						
18-19	16	10.6	27	16.9	43	13.8
20-21	54	35.8	74	46.2	128	41.2
22-23	44	29.1	46	28.8	90	28.9
24 ve over	37	24.5	13	8.1	50	16.1
Mean	22.02±2.38		21.25±2.09		21.62±2.26	
Student's class						
Freshmen	31	20.5	31	19.4	62	19.9
Sophomore	28	18.5	49	30.6	77	24.8
Junior	45	29.8	47	29.4	92	29.6
Senior	47	31.2	33	20.6	80	25.7
Student's residence						
Dormitory	56	37.1	112	70.0	168	54.0
Shares a flat with other students	43	28.5	11	6.9	54	17.4
With his/her family	47	31.1	37	23.1	84	27.0
With relatives	5	3.3	0	0	5	1.6
Mother's education level						
Illiterate	7	4.6	8	5.0	15	4.8
Primary school	105	69.6	123	76.9	228	73.3
High school	26	17.2	18	11.2	44	14.2
Graduate and uppergraduate	13	8.6	11	6.9	24	7.7
Father's education level						
Illiterate	2	1.3	1	0.6	3	1.0
Primary school	67	44.4	72	45.0	139	44.7
High school	43	28.5	52	32.5	95	30.5
Graduate and uppergraduate	39	25.8	35	21.9	74	23.8
Family's residence place						
Village	11	7.3	11	6.9	22	7.1
Town	5	3.3	12	7.5	17	5.5
City	135	89.4	137	85.6	272	87.5

Examination of students' milk consumption

It was determined that most of the students (85.5%) in the study consumed milk. According to this finding, it can be argued that university students representing the young population exhibit a positive consumption behavior for a healthy diet. However, in this study students milk consumption amounts were not measured, which is a significant limitation of the study. In another study, it was determined that although 78.96% of the students liked drinking milk, they did not drink enough milk [7]. Contrary to the current study, some studies found that the rate of students who drink milk is low. For example, in their study Yılmaz and Özkan [9] reported that only 5.1% of the students drank milk. In a study on university students in Istanbul, it was determined that 28.0% of them drank fizzy drinks every day and 67.7% generally drank milk [10]. In a study by Çetinkaya [11] on university students, it was reported that 33.0% consumed drinking milk, and 67.0% did not consume milk. In the present study, milk consumption rate (85.5%) is higher than that in the mentioned studies, which is gratifying.

Although the number of those who live in city centers make up most of the group, milk consumption rates of those whose families live in the vil-

lages were found to be lower compared to those in towns and cities ($p<0.01$) (Table 2). This can be attributed to the fact that students from villages who attend universities in the city centers are not used to consuming milk as their families in villages generally produce the milk and milk products themselves and/or because they find packaged milk expensive. Contrary to the current study, in a study by Ayar and Demirulus [12], 45.2% of the university students liked drinking milk and most (79.2%) of those who liked drinking milk were of village origin.

As the essential value of milk is its dry matter, knowing its amount in milk is technologically of great importance. Under normal conditions, milk's nutritive value and its efficiency when it is processed as milk products depends on its dry matter [13]. Therefore, when Table 2 is examined, 39.8% of the students preferred skimmed milk, which is followed by whole milk (29.7%), fat (26.3%) and no-fat milk (4.1%). In other words, more than half of the students (56.0%) consumed whole-fat milk. According to 2010 World and Turkish Milk Industry Report, most of the drinking milk is whole-fat milk [6]. Similarly, in another study, 42.7% of the students preferred fat milk, 27.2 % preferred semi-skimmed milk [11].

On the other hand, the reason why skimmed milk and no fat milk are preferred can be that it is cheaper and/or it does not include more calories. Besides, as education levels of parents decreased, the level skimmed milk preferred increased ($p<0.05$). In this case, low-fat milk preference can be linked with low socio-economic level.

In a study by Tarakçı et al [7], it was revealed that 19.13% of the students preferred whole milk, 28.69% preferred fat milk, 44.54% preferred low-fat milk, and 7.65% preferred no fat milk. Therefore, a little more than half of the students (52.19%) consumed no-fat milk, almost half (47.82%) consumed whole milk. In a study by Özel [14] in which milk preferences of consumer in Ankara, it was determined that semi-skimmed milk was preferred. In the mentioned study, the fact that skimmed milk was preferred was attributed to high education level and high number of women probably not wanting to take high calorie contrary to the attribution to economic inadequacy in the present study. In a study by Yıldız [15] on 6 to 14 year olds milk consumption, it was reported that 34.7% of them consumed whole milk, 48.1% consumed low-fat milk. In the same study, it was seen that 17.2% of the students did not know the fat rate of the milk they consume. Therefore, the last three studies support the findings of the current study.

Milk, which has a significant place in the food sector, is processed, stored and distributed using various technologies so that it does not spoil in short time. There are two production methods: pasteurization and sterilization. Pasteurized milk, also known to be pasteurized milk, is to be preserved in cool places including the transportation and is to be consumed within two days after its production. Sterilized milk, also known as long life milk or UHT-treated milk is obtained by eradicating all organisms that can cause spoiling with UHT process with the least change in chemical and physical properties of raw milk and filled in sterilized package under aseptic conditions. Therefore, sterilized milk can endure longer than pasteurized milk [14]. Farm milk which is also called as street milk in Turkey is subject to vitamin loss as it is boiled at home. For example, in a study on unprocessed milk it was determined that vitamin values were lower than expected. It was determined that boiling milk for ten minutes lead to significant losses in tiamin, riboflavin, niasin, B_{12} and folic acid vitamins at 60%, 25%, 12%, 21% and 32% rates, respectively and these losses increased even more when it is boiled for 15 minutes (66%, 34%, 12%, 28% and 50%). As a result of this study, it was emphasized that boiling at homes is to be limited with five minutes to minimize vitamin losses, but it was also stated that five minutes would not be enough to eradicate microorganism that can cause some diseases [16]. Therefore, the consumption of street milk, which seriously threatens public health, is to be abandoned. This case imposes serious obligations on the milk industry. The main reason why street milk is used is its low price. Milk industry is to decrease the cost of processes applied and increase the use of UHT milk technology [14].

In the study, 32.8% of the students consumed pasteurized milk, 27.9% preferred sterilized (UHT) milk, 14.0% consumed farm milk and 25.3% stated that it did not matter for them (Table 2). The relation between students' milk consumption preferences and their mothers education level ($p=0.03$). When other studies on this issue are ex-

amined, the rate students prefer farm milk in different regions were 9.5% [1], 13.4% [7], 17.6% [11] and similar to that obtained in this study. The rate pasteurized milk preferred according to other studies are 50.4% [17], and 60.5% [1] and higher that found in the present study. The rate sterilized milk preferred were found to be 40.7% [17], 30.0% [1] and 71.5% [11] and varied along studies. Given that pasteurized milk, which is cheaper but short-lived compared to UHT milk, cannot be found in every supermarket, the fact that in some studies pasteurized milk in some studies sterilized milk is preferred more can be attributed to supermarket preferences and regional differences.

In studies on families' milk consumption levels, regional differences matter, while consumers in urban areas in Şanlıurfa province 46.3% [18], in Kahramanmaraş province 33.9% of families [19] and 67.8% of the families in Erzincan [20] prefer farm milk, 11% of consumers in Istanbul prefer farm milk, 49% prefer pasteurized milk, 40% prefer sterilized milk [21]. As for Ankara, it was determined that consumers prefer daily milk (pasteurized milk) to sterilized milk [14]. Therefore, as we go to the west of the country, people prefer street milk less and are more conscious about milk consumption, and as we go to the east people are less conscious about this issue. Similarly, in a study by Özmetin [22] in which milk consumption habits of people in Düzce, Bursa, Istanbul and Sakarya provinces, which represent the west of the country, for the previous five years between 2000 and 2004, it was observed that package milk consumption increased 54%. In the mentioned studies it was also determined that people's package milk consumption increased as their income [19] and education levels increase [22]. However, processed and package milk consumption in Turkey falls rather behind compared to other European countries. For example, while in Turkey average package milk consumption per capita is 6 liters; it is 139 liters in Finland, 108 liters in Spain, and 100 liters in the UK and 65 liters in Greece [20].

When students' milk consumption is examined according to the flavor they prefer, 83.5% prefer unflavored, 13.5% prefer flavored (cacao, banana flavor etc.) and 3.0% prefer it with coffee (Table 2). Besides, there found no statistically significant relation between milk flavor preference and demographic characteristics of the students ($p>0.05$) (Table 2). Similarly, in other studies it was revealed that mostly unflavored milk was preferred [20; 22; 23]. In another study, it was observed that flavored milk was preferred more by 7 to 15 year olds [20], in another study it was revealed that flavored milk was preferred between ages 16 and 25 [22].

When the students' preferences of milk products instead of milk were examined, it was revealed that 61.4% prefer milk products, 38.6% prefer milk. Similarly, in a study by Hanta and Yurdakul [24] it was determined that 15.1% of the food expenses of households in Adana province was allocated to milk and 51.8% was allocated to milk products. In another study, 75.9% of the students answered positively when asked "Do you prefer milk products instead of milk?" and most of them consumed milk products instead of milk [11]. As a matter of fact, according to 2010 World and Turkey Milk Industry Report in our country milk is mostly consumed as yoghurt, white cheese and buttermilk [6]. When the relation between the mentioned preference and demographic variables is examined, it was revealed that while those whose mothers are primary school graduates (68.0%) prefer milk, those whose mothers have less or more education level mostly prefer milk products.

In Table 2, it is see that students mostly (74.3%) purchase milk and milk products from supermarkets, 12.9% did not have any certain preference about the place they purchase milk, 8.0% produced their own milk and milk products and 4.8% bought from groceries. Similarly it was determined that students mostly purchased dirking milk from supermarkets in other studies by Tarakçı et al [7] (60.64%), Uzunöz and Gülşen [8] (93.33%) and Mortaş et al [1] (81.40%). Besides, in the present study it was revealed that the place students buy milk and milk products were found to be their fathers' education level and the place their families live. The rate those whose fathers were high school and primary school (5 years) graduates produce milk and milk products themselves was found to be higher than that of those whose fathers were primary school (8 years) graduates ($p<0.05$). As expected the students whose families live in city centers mostly purchase milk and milk products from supermarkets (79.8%), the student whose families live in villages (40.9%) and town

centers (41.2%) mostly produce their own milk and milk products ($p<0.001$).

The students in this study it was determined that primarily prefer factory/diary milk products (37.3%), which is followed by village/homemade products (33.1%), while for 29.6% it does not matter (Table 2). Contrary to expectations, while those whose families live in villages (59.1%) and in towns (41.2%) prefer milk products produced by factories or diaries, those whose families live in city centers (40.1%) mostly prefer home-made products or products produced in villages ($p<0.05$). This can be attributed to the fact that the students who live in city centers yearn for natural products as the products they generally purchase are processed and include additives. The processed products might have been more attractive to those who live in villages and towns as they generally produce their own products. However, the quality of the milk consumed is as much important as the amount of the milk consumed because besides useful nutritive substances the milk composition provides an environment where organisms that are harmful for people can proliferate. Therefore, right after the milk is produced, both drinking milk and milk products are to be presented to consumers [18].

This case can stem as much from the socio-economic and demographic characteristics of consumers like income, education level, gender, household number as from the scarcity of modern enterprises [20]. When the case is internationally examined 53% of the milk produced all over the world is processed in modern milk dairy plant. This rate is 94% in the EU. In developed countries milk breastfed to animals or lost is 2 to 3 % and 1% to 5% of the milk is consumed in the places where they are produced and the remaining 95% to 99% is used in the milk industry. When the distribution of the raw milk in country is examined, 25% of raw milk is consumed in the production units, 10% is breastfed to baby animals, 5% is lost and thus only 60% of the total amount of raw milk can be marketed. 40% of the milk marketed reaches to consumers unprocessed, 10% are processed in modern plants, and 10% is processed in small and simple plants called dairy. Thus the rate raw milk is reached to the industry is rather lower than that in developed countries. When we add more than 40% off the books milk consumed, it can be said that 60% of the families consume farm milk, which is called street milk or open milk in Turkish [6; 20].

Finally, in Table 2 the views of the students about the prices of milk products were examined. Most of the students (72.0%) found the price of milk normal, 25.1% regard them expensive and 2.9% regarded them cheap. No statistically significant relation between students' views about the prices of milk products and demographic variables was found ($p>0.05$). In other studies, students were directly asked whether they find the prices expensive or cheap. In some of these studies, exactly half of the students found milk expensive (50.0% [7]; %51.42 [23]), in other studies almost half of the students (49.90% [1]; 53.09% [17]) found the price of milk reasonable. Therefore, the data from the last two studies comply with the findings of the present study.

Examination of criteria students use when purchasing milk and milk products

the date of production and expiry date, storage conditions, nutritive values and their net amounts are some of the important information that are to be present on food labels. Many producer firms use production and expiry date labeling printing that cannot be deleted, removed or changed. However, small enterprises still from problems in production date and expiry date printing. Consumers' habit of checking this information on food packages is another important issue [25].

In this study, the criteria that the students mostly pay attention to when purchasing milk and milk products was found to be expiry date (80.4%). It was even revealed that female students are more sensitive to this issue compared to male students ($p<0.05$) (Table 3). Similarly, while in many studies expiry date was found to be the criterion to which people pay the most attention when purchasing milk and milk products [1; 7; 8; 25], in a study it was found to be in the second most important criterion [26], and yet another study determined that expiry date was always paid attention at 44.3% and sometimes paid attention at 54.2% [20]. Still another study reported that it was paid attention at only 18.4% [18].

As it is seen in Table 3, the second most important criterion the students pay attention when purchasing milk and milk products was found to be the price with 71.4%, which was not found to be

Table 2. The distribution of students' milk consumption condition with independent variables

		f	%	Student's Gender 1. Female 2. Male	Student's Age 18-19 20-21 22-23 24 and over	Student's Grade Freshmen Sophomore Junior Senior	Student's Residence 1. Dormitory 2. Sharing a flat with other students 3. Family 4.With Relatives	Mother's Education Level 1. Illiterate 2. Primary School 3. High School 4. Undergraduate and over	Father's Education Level 1. Illiterate 2. Primary School 3. High School 4. Undergraduate and over	The place the family lives 1. Village 2. Town 3. City
Drinking milk consumption (n=311)	1. Consume 2. Does not consume	266 45	85.5 14.5	χ^2=0.481 sd= 1 p= 0.488	χ^2= 1.073 sd= 3 p=0.784	χ^2= 0.998 sd= 3 p= 0.802	χ^2= 1.421 sd= 3 p= 0.701	χ^2= 0.892 sd= 3 p= 0.827	χ^2= 1.964 sd= 3 p= 0.580	χ^2= 14.004 sd= 2 p= **0.001****
The rate of fat preferred in milk (n=266)	1. Whole fat milk 2. Fat milk 3. Low fat milk 4. Fat free milk	79 70 106 11	29.7 26.3 39.8 4.1	χ^2= 0.993 sd= 3 p= 0.803	χ^2= 3.759 sd= 9 p= 0.927	χ^2= 5.766 sd= 9 p= 0.763	χ^2= 9.448 sd= 9 p= 0.397	χ^2= 17.121 sd= 9 p= **0.047***	χ^2= 8.023 sd= 9 p= 0.532	χ^2= 4.890 sd= 6 p=0.558
The most preferred milk life (n=266)	1. Farm milk 2.Sterilized (UHT) milk 3. Pasteurized milk 4. Does not matter	37 74 87 68	13.9 27.8 32.7 25.6	χ^2= 4.366 sd= 4 p= 0.359	χ^2= 13.195 sd= 9 p= 0.154	χ^2= 12.161 sd= 9 p= 0.204	χ^2= 11.430 sd= 9 p= 0.247	χ^2= 17.726 sd= 9 p= **0.038***	χ^2= 6.726 sd= 9 p= 0.666	χ^2= 4.606 sd= 6 p= 0.595
Flavour preference (n=266)	1. Unflavored milk 2. Flavored (Cacao, banana, etc.) milk 3. Milk with coffee	222 36 8	83.5 13.5 3.0	χ^2= 0.020 sd= 2 p= 0.990	χ^2= 6.765 sd= 6 p= 0.343	χ^2= 8.262 sd= 6 p= 0.220	χ^2= 3.842 sd= 6 p= 0.698	χ^2= 5.498 sd= 6 p= 0.482	χ^2= 11.759 sd= 6 p= 0.068	χ^2= 1.763 sd= 4 p= 0.779
Preferring Milk products instead of milk (n= 311)	1. Prefer 2. Does not prefer	191 120	61.4 38.6	χ^2= 7.482 sd= 1 p= 0.006	χ^2= 5.467 sd= 3 p= 0.141	χ^2= 2.890 sd= 3 p= 0.409	χ^2= 4.361 sd= 3 p= 0.225	χ^2= 16.324 sd= 3 p= **0.001****	χ^2= 4.737 sd= 3 p= 0.192	χ^2= 0.490 sd= 2 p= 0.783
The place they purchase milk and milk products (n=311)	1. Supermarket 2. Grocer 3. Self-production 4. Does not matter	231 15 25 40	74.3 4.8 8.0 12.9	χ^2= 3.545 sd= 3 p= 0.315	χ^2= 6.111 sd= 9 p= 0.729	χ^2= 4.021 sd= 9 p= 0.910	χ^2= 9.571 sd= 9 p= 0.386	χ^2= 9.322 sd= 9 p= 0.408	χ^2= 19.552 sd= 9 p= **0.021***	χ^2= 71.514 sd= 6 p= **0.0001*****
Preference of the place milk is processed (n= 311)	1. Home/village made 2. Dairy/factory made 3. Does not matter	103 116 92	33.1 37.3 29.6	χ^2= 2.134 sd= 2 p= 0.334	χ^2= 6.544 sd= 6 p= 0.365	χ^2= 5.345 sd= 6 p= 0.500	χ^2= 3.569 sd= 6 p= 0.735	χ^2= 8.707 sd= 6 p= 0.191	χ^2= 7.655 sd= 6 p= 0.264	χ^2= 10.949 sd= 4 p= **0.027***
Their views of milk products' prices (n=311)	1. Expensive 2. Normal 3. Cheap	78 224 9	25.1 72.0 2.9	χ^2= 1.238 sd= 2 p= 0.538	χ^2= 7.553 sd= 6 p= 0.273	χ^2= 7.682 sd= 6 p= 0.262	χ^2= 1.613 sd= 6 p= 0.952	χ^2= 9.756 sd= 6 p= 0.135	χ^2= 3.511 sd= 6 p= 0.742	χ^2= 7.253 sd= 4 p= 0.123

*$p < 0.05$ **$p < 0.01$ ***$p < 0.001$

not related with any of the demographic characteristics (p>0.05). However, the fact that students pay attention to the price of the milk indicates that they care about milk price and question whether they are at suitable prices for their budget. Contrary to the present study, the price of the milk was found to be the third criterion used when buying milk in the study by Sütütemiz et al., [26]. In a study by Tarakçı et al., [7], it was found to be on the fourth position and in Candemir's [19] study it was in the last position. In their study Çelik et al., [18], although brand was regarded to be an effective factor only by 17.5% of participants, cheapness was found to be the third important factor in choosing brand with 64.8%.

In this study, the third criterion the students use when purchasing milk and milk products was found to be brand with 62.4% (Table 3). Similarly, in a study by Özel [14] it was found that the brand of the milk and milk products was in the third position among purchasing criteria (22.4%). It was found to be in the second position in Tarakçı et al.'s [7] study and in Mortaş et al.'s [1] study it was in the second position after expiry date, in a study by Sütütemiz et al., [26] it was in the fifth position. When other studies are examined, in some studies more than half of the participants (56.2%) [18], in some studies most of them (70.33%) [8], (74.5%) [11], in another study 27.5% [20] stated that they paid attention to brand when buying milk. In short, all of the studies mentioned above reveal that consumers paid attention to the brand of the milk and milk products. Besides, in this study it was revealed that students pay attention to the brand of the milk and milk products depends on their ages (p<0.001). It was determined that those aged between 20 and 23 paid more attention to the brand of the milk and milk products compared to those younger than 20 and older than 23.

Besides, according to Table 3, more than half of the students (57.2%) did not pay attention to hygiene when buying milk and milk products. It was even observed that boys were less sensitive to this issue compared to girls (p<0.05). By the same token, in a study by Tarakçı et al [7], the percent of those who are sensitive to hygienic quality when buying milk was found to be as low as 16.0%. On the contrary, in a study by Uzunöz and Gülşen [8] it was found that 73.67% of the students paid attention to hygiene. In a study by Erdal and Tokgöz [20] it was emphasized that among the reasons why consumers prefer certain brands when buying milk reliability and hygiene were the leading factors, which accounts for 30.4% of preference.

The amount of fat in milk was found to be the fifth criterion students use when buying milk and milk products and 41.8% of the students reported that they paid attention to fat amount. At the same time, this criterion was not found to be related with demographic characteristics (p>0.05) (Table 3). When other studies on this issue were examined, the percent consumers pay attention to fat rate when purchasing milk was 7.9% in Çelik et al.'s (18) study in Uzunöz and Gülşen's (8) study it was 19.66%; in Özel's (14) study it was 27.7%. When compared with these studies, in the present study the percent of the students who paid attention to fat rate when purchasing milk is rather high (41.8%), which can be taken to mean that the public has become more conscious about this issue over the period of time.

Interestingly, it was determined that most of the students in the study paid attention neither the quality certification of the milk and milk products (87.1%) nor whether there were different products of the same type available in the supermarket (92.9%). Moreover, theses criteria were not found to be related with any of the demographic characteristics (p>0.05) (Table 3). This can be attributed to the fact that students prefer a certain brand and rely on that brand and thus they do not investigate whether there are the same products with different brands. The students who bought "open milk" (farm milk) (14.0%) or those who stated that it did not matter whether it had a package (25.3%) are naturally not expected to pay attention to these criteria.

Results and Sugesstions

It was determined that 85.5% of the university students in the study consume milk, and that 83.5% consumed unflavored milk and 56.0% consumed fat and whole fat milk, 32.8% drank pasteurized milk, 27.9% drank sterilized milk, 14.0% drank farm milk. Besides, it was revealed that 61.4% of the students prefer milk products instead of milk. It was also determined that they mostly (74.3%) milk and milk products from supermarkets. They primarily prefer

Table 3. Distribution of the relations between the criteria students use when purchasing milk and milk products and independent variables (n= 311)

The criteria observed when buying milk and milk products		f	%	Student's Gender 1. Female 2. Male	Student's Age 18-19 20-21 22-23 24and over	Student's Grade Freshmen Sophomore Junior Senior	Student's Residence 1. Dormitory 2. Shares a flat with other students 3. With his/her family 4. With relatives	Mother's Education Level 1. Illiterate 2. Primary school 3. High school 4. Undergraduate and graduate	Father's Education Level 1. Illiterate 2. Primary school 3. High school 4. Undergraduate and graduate	Family's Residence Place 1. Village 2. Town 3. City
1. Expiry date	Cares about	250	80.4	χ^2= 4.450 sd= 1 **p= 0.035***	χ^2= 2.578 sd= 3 p= 0.461	χ^2= 1.421 sd= 3 p= 0.701	χ^2= 5.436 sd= 3 p= 0.143	χ^2= 2.321 sd= 3 p= 0.508	χ^2= 3.458 sd= 3 p= 0.326	χ^2= 5.946 sd= 2 p= 0.051
	Does not care about	61	19.6							
2. Price	Cares about	222	71.4	χ^2= 0.490 sd= 1 p= 0.484	χ^2= 0.666 sd= 3 p= 0.881	χ^2= 2.218 sd= 3 p= 0.528	χ^2= 2.929 sd= 3 p= 0.403	χ^2= 3.144 sd= 3 p= 0.370	χ^2= 3.922 sd= 3 p= 0.270	χ^2= 1.648 sd= 2 p= 0.439
	Does not care about	89	28.6							
3. Brand	Cares about	194	62.4	χ^2= 3.682 sd= 1 p= 0.055	χ^2= 19.768 sd= 3 **p= 0.000****	χ^2= 5.698 sd= 3 p= 0.127	χ^2= 2.295 sd= 3 p= 0.514	χ^2= 5.246 sd= 3 p= 0.155	χ^2= 1.635 sd= 3 p= 0.651	χ^2= 1.584 sd= 2 p= 0.453
	Does not care about	117	37.6							
4. Hygiene	Cares about	133	42.8	χ^2= 3.868 sd= 1 **p= 0.049***	χ^2= 4.390 sd= 3 p= 0.222	χ^2= 1.515 sd= 3 p= 0.679	χ^2= 4.840 sd= 3 p= 0.184	χ^2= 6.746 sd= 3 p= 0.080	χ^2= 4.948 sd= 3 p= 0.176	χ^2= 3.660 sd= 2 p= 0.160
	Does not care about	178	57.2							
5. Fat rate	Cares about	130	41.8	χ^2= 0.515 sd= 1 p= 0.473	χ^2= 1.068 sd= 3 p= 0.785	χ^2= 3.573 sd= 3 p= 0.311	χ^2= 5.362 sd= 3 p= 0.147	χ^2= 3.191 sd= 3 p= 0.363	χ^2= 2.953 sd= 3 p= 0.399	χ^2= 0.011 sd= 2 p= 0.994
	Does not care about	181	58.2							
6. Quality certificate	Cares about	40	12.9	χ^2= 0.009 sd= 1 p= 0.926	χ^2= 1.861 sd= 3 p= 0.602	χ^2= 3.175 sd= 3 p= 0.365	χ^2= 3.779 sd= 3 p= 0.286	χ^2= 2.422 sd= 3 p= 0.489	χ^2= 2.783 sd= 3 p= 0.426	χ^2= 1.481 sd= 2 p= 0.477
	Does not care about	271	87.1							
7. Diversity	Cares about	22	7.1	χ^2= 0.091 sd= 1 p= 0.763	χ^2= 2.120 sd= 3 p= 0.548	χ^2= 3.535 sd= 3 p= 0.316	χ^2= 0.935 sd= 3 p= 0.817	χ^2= 3.513 sd= 3 p= 0.319	χ^2= 3.490 sd= 3 p= 0.322	χ^2= 1.564 sd= 2 p= 0.457
	Does not care about									

*$p < 0.05$ **$p < 0.01$ ***$p < 0.001$

milk products produced by plants/dairies (37.3%), which were followed by home/village made milk products (33.1%), most of them (72.0%), found the prices of milk products reasonable.

It was determined that the students mostly (80.4%) pay attention to expiry date when buying milk and milk products, which is followed by the price (71.4%) and brand (62.4%). Besides, more than half o the students (57.2%) and fat rate (58.2%), and that most of them did not care about the existence quality certificate (87.1%) and variety of products (92.9%).

As it can be seen in the data above, it was determined that only 60.7% of the university students consumed packaged milk. This is because of the lack of conscious among consumers about processed milk. In Turkey, many consumers regard packaged long life milk as "dead" milk due to heat treatment during their production. Some consumers believe that antibiotic and antiseptic substances are added to packaged long life milk during treatment and that the package materials cause cancer. Therefore, majority of the consumers regard "street milk" (farm milk) as "the purest", "most natural" and "freshest" milk (20). As a matter of fact, in a study by Erdal and Tokgöz [20], while almost 70% of the consumers consider that farm milk is healthy only 13% did not agree with this and 17% stated that they did not have any idea about this issue. However, milk is a complex food product and includes many nutritive elements. However, although these nutritive elements may be very beneficial for human health, farm milk (if it is not treated with heat) it can threat human health as it is a good nutritive and proliferation environment for many organisms [27].

In this case, both milk producers and package producers are to lie heavy on ads and promotion activities to increase public's consumption of packaged milk, which is safe for public's health. Furthermore, non-governmental organization and media are to organize awareness rising and education activities to prevent the consumption of farm milk, which seriously threatens public health. Besides, local authorities are to strengthen auditing mechanism to control the sale of "street milk", which is off the books. It is also important that these audits be made by experts and regularly. The milk industry is to generalize UHT milk technology by decreasing the cost of treatments. Besides, the industry is to increase distribution points to make the availability of pasteurized milk easier and thus make it competitive with the "street milk".

Thus, pasteurized milk and UHT milk with various quality and flavors will be available in the supermarkets, which will enable every consumer in every part of Turkey to use the "Right for Choosing Goods and Services Freely". This right means that consumers can prefer cheap and quality goods or services as they please according to their income levels. In other words, this right includes the presentation of goods and services with adequate price and quality variety [28; 29]. It is hoped that the availability of pasteurized and sterilized milk varieties at different price and quality in every supermarkets will also increase the consumption of milk consumption rates of our people.

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The effect of repetitive passive heat loading on the adipoinsular axis response in humans

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Abstract

Objective: The purpose of this study was to determine whether the adipoinsular axis reaction is closely related to energy balance.

Methods: Subjects who underwent repetitive passive heat loading (PHL, half bath in warm water at 43°C) for 4 weeks (three times/week, 30 min/day; n = 15, age, 24.37 ± 3.15 yrs; height, 172.61 ± 3.74 cm; weight, 67.92 ± 4.80 kg; body fat, 19.85 ± 3.84%; muscle mass, 20.79 ± 4.26 kg).

Results: The results showed that regular PHL for 4 weeks significantly inhibited insulin expression (Pre time-point: before vs. after, $p < 0.001$; Post time-point: before vs. after, $p < 0.01$), and leptin level decreased significantly following the adipoinsular axis cascade (Pre time-point: before vs. after, $p < 0.01$; Post time-point: before vs. after, $p < 0.01$). Free fatty acids increased significantly (Post time-point: before vs. after, $p < 0.01$) based on this reaction. **Conclusion:** It is concluded that the adipoinsular axis reaction was caused by activation of the sympathetic nervous system, once subjects experienced PHL. PHL contributed to inhibit insulin and leptin expression independently of the adipoinsular axis reaction by decreasing the amount of accumulated and visceral fat simultaneously.

Key words: Passive heat loading, Adipoinsular axis, Insulin, Leptin, Sympathetic nervous system

Introduction

The site of fat accumulation has a major influence on human health, as described by the relationship between type II diabetes mellitus (DM) and obesity. High insulin sensitivity and peripheral obesity, in which subcutaneous fat accumulates in the hip and leg regions, significantly lowers health risk compared with central obesity, in which visceral fat accumulates [1].

However, central obesity causes negative metabolism when visceral fat is removed by omentectomy, and insulin decreases [2]. However, no significant improving effect on metabolic syndrome is observed by removing subcutaneous fat via liposuction [3].

Central obesity increases insulin resistance, leading to type II DM, dyslipidemia, and atherosclerosis and can result in death [4, 5], indicating that improving insulin sensitivity through diet or exercise is more closely related to reducing visceral fat rather than reducing subcutaneous fat because central or visceral fat is a major factor determining insulin sensitivity and serum insulin levels [6].

Insulin inhibits appetite by acting on the hypothalamus and repressing neuropeptide Y expression [7]. Administering insulin in a controlled manner that does not lead to hypoglycemia results in decreased consumption of food [8], whereas activating the sympathetic nervous system (SNS) increases thermogenesis [9].

Leptin is considered a component of the adipoinsular axis, as it is related to the insulin negative feedback loop [10].

Energy consumption increases when leptin binds receptors in the central nervous system due to increased lipolysis and body heat production and activation of liver and skeletal muscle metabolism by activation of the SNS [11, 12].

An experiment on rats fed a high-fat diet and treated with heat loading once per week (half body water immersion at 41°C for 20 minutes) showed that increased peripheral insulin resistance can be prevented [13], suggesting an influence on the adipoinsular axis reaction, together with changes in insulin expression and a potentially favorable effect on lipid metabolism and energy consumption.

Although relevant studies have been conducted actively in Korea [14-18], no studies related to the adipoinsular axis have been reported. Therefore,

in this study, we investigated the effect of repetitive passive heat loading (PHL, half bath up to umbilical line with constant-temperature water at 43°C for 30 minutes) on the bidirectional feedback loop, i.e., the adipoinsular axis 12 times over 4 weeks by identifying the change in insulin and leptin levels, total body sweating, blood free fatty acids (FFA) level, and waist size (WS).

Methods

Subjects

The subjects were 15 males in their 20s who resided in Cheonan (126º52'N, 33.38'E) and who had not experienced intentional, habitual, or repetitive heat loading. The physical characteristics of the subjects are listed in Table 1. Subjects were notified in advance not to take any substance that might affect body fluid balance, such as alcohol, caffeine, or diuretics 24 hours prior to PHL, and food was restricted 2 hours before the test. The significance and purpose of this study and all risks related to the experimental procedures were fully explained to the subjects before testing. This study was approved by the Ethics Committee of Suncheonhyang University, and the procedures complied with the 1975 Declaration of Helsinki, as revised in 1983.

Table 1. Participant characteristics

Variables	Means±SD
Age (yrs)	24.37±3.15
Height (cm)	172.61±3.74
Weight (kg)	67.92±4.80
Muscle mass (kg)	20.79±4.26
(%) Body fat	19.85±3.84
BMI	21.06±2.45
VO_{2max}($ml \cdot kg^{-1} \cdot min^{-1}$)	44.73±6.97

Values are mean ± standard deviation (SD).

Measurement procedures

This study was conducted in a climate chamber from 2–5 p.m., and the environmental conditions were maintained at 24.5 ± 0.3°C, 50 ± 3.0% relative humidity, and 1 m/second air velocity. Any subtle interpersonal variability in human body temperature would range from 36.6 ± 0.5°C, and usually body temperature is at its lowest at 4 a.m., and highest from 4–6 p.m. [19]. Thus, we conducted this experiment from 2–5 p.m. to control for the influence of the body temperature circadian rhythm, as described previously [14, 15, 17, 20-22]. PHL was performed after a 2 hour fast. After the subjects arrived in the laboratory, urine specific gravity was tested with a urine strip (Uriscan, Seoul, Korea) to confirm hydration equilibrium. The test results were confirmed by visually inspecting the color change on the strip . Tests were delayed in cases in which the test strip color change exceeded the reference range of 1.010–1.025 [23].

Subjects were dressed in short sleeves and shorts and asked to take a 60 minute rest in the sitting position in the laboratory. Every participant was asked to drink 200 ml of tap water before the PHL on the day of the study to control liquid ingestion from the beginning to the end of the test. Blood sampling and measurements of body weight and WS were obtained, the subjects removed their shirts, and PHL was performed for 30 minutes. Body weight and WS were measured, and blood was sampled after completion of PHL

PHL

PHL was performed via the half bath method in hot water (constant temperature of 43 ± 0.3°C). PHL was performed 12 times every other day for 4 weeks (three times/week, 30 minutes/day). The testing was performed in June 2011, and the mean external environmental temperature, relative humidity, and air velocity were 21.98 ± 2.63°C, 66.56 ± 16.15%, and 2.92 ± 1.08 m/s, respectively.

Mean whole body sweat loss volume and WS

As the decrease in weight observed immediately after PHL was primarily due to sweating, mean whole body sweat loss volume was assessed based on the change in weight before and after the test. WS was measured at the same time as body weight. Subjects were asked to lightly exhale in the erect position before WS was measured. WS was measured between the lowest rib and the highest portion of the iliac crest using a tape placed horizontally without artificial pressure exerted on the waist skin. WS was measured by skilled personnel to within 0.1 cm [24].

Blood sampling and analysis

Five ml blood samples were collected four times from the antecubital area using a venipuncture kit and SST tube. Blood was collected before (Pre) and after (Post) the initial 30 minute PHL, and before and

after an additional 30 minute PHL and after the subjects had completed the 4 weeks of scheduled tests. Blood samples were left at room temperature for 30 minutes, and centrifuged for 10 minutes at 2000 × g. After the serum was separated, the levels of insulin (E 170, Modular Analytics, Roche, Mannheim, Germany), leptin (gamma-counter Cobra 5010, Quantum, Packard, Dallas, TX, USA), and FFA (Hitachi, Hitachi 7180, Tokyo, Japan) were analyzed.

Statistical analysis

All data are expressed as mean ± standard deviation. The paired *t*-test was used to test differences in insulin, leptin, and FFA levels before and after PHL. P-values < 0.05 were considered statistically significant.

Results

None of the subjects experienced any side effects that were related to PHL.

Insulin

As shown in Figure 1, a significant decrease in serum insulin level was found after rather than before the 4 weeks of repetitive PHL (Pre time-point: before vs. after, 14.52 ± 4.25 vs. 9.40 ± 4.21 μU/mL, p < 0.001) (Post time-point: before vs. after, 10.20 ± 3.71 vs. 6.71 ± 2.71 μU/mL, p < 0.01).

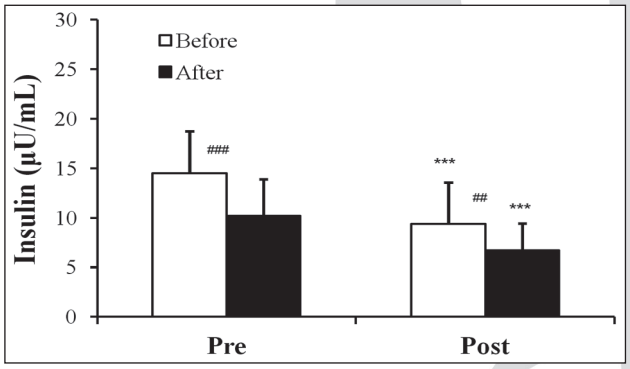

*Figure 1. Serum insulin levels before and after repetitive passive heat loading. Values are mean ± standard deviation. Significantly different at Pre time-point: before vs. after at ###p<0.001 and Post time- point: before vs. after, at ##p<0.01, and significantly different between Pre and Post at ***p<0.001, respectively.*

Before, before experiencing repetitive passive heat loading for 4 weeks; After, after experiencing repetitive passive heat loading for 4 weeks; Pre, before passive heat loading for 30 min; Post, after passive heat loading for 30 min.

Mean whole body sweat loss volume

Mean whole body sweat loss volume increased significantly after PHL compared with that before (before vs. after, 651±157 vs. 749±184 ml, p<0.001).

FFA

No significant change was found in the FFA levels before and after at the Pre time point (before vs. after, 265.03 ± 52.14 vs. 253.17 ± 62.19 μEq/L), but significantly higher serum FFA level was observed 30 minutes after PHL and after subjects had completed the 4 weeks of PHL (Post time-point: before vs. after, 375.52 ± 115.70 vs. 472.97 ± 131.45 μEq/L; p < 0.01) (Figure 2).

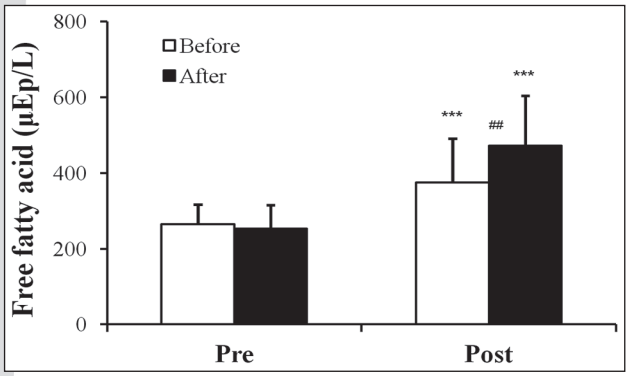

*Figure 2. Serum free fatty acid levels before and after repetitive passive heat loading. Values are mean ± standard deviation. Significantly different at Post time-point: before vs. after at ##p < 0.01, and significantly different between Pre and Post at ***p < 0.001, respectively.*

Before, before experiencing repetitive passive heat loading for 4 weeks; After, after experiencing repetitive passive heat loading for 4 weeks; Pre, before passive heat loading for 30 min; Post, after passive heat loading for 30 min.

WS

WS decreased significantly after 4 weeks of repetitive PHL compared with that before the test (before vs. after, 7.4 ± 2.3 vs. 9.5 ± 2.6 mm; p < 0.001).

Leptin

As shown in Figure 3, a significant decrease in serum leptin level was found after rather than be-

fore the 4 weeks of repetitive PHL (Pre time-point: before vs. after, 51.26 ± 15.75 vs. 41.13 ± 13.25 ng/mL; $p < 0.01$) (Post time-point: before vs. after, 41.46 ± 14.85 vs. 32.20 ± 12.10 ng/mL; $p < 0.01$).

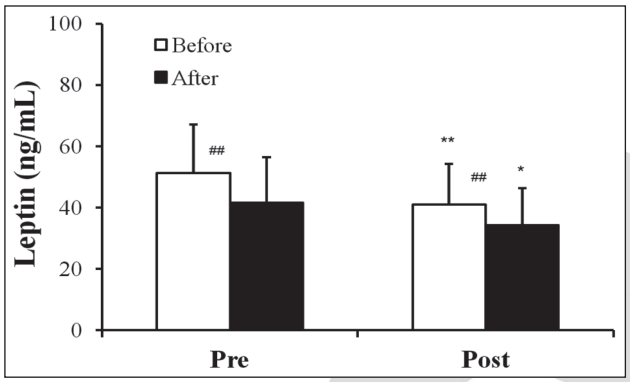

Figure 3. Serum leptin levels before and after repetitive passive heat loading. Values are mean ± standard deviation. Significantly different at Pre time-point: before vs. after, $^{\#\#}p < 0.01$ and Post time-point: before vs. after at $^{\#\#}p < 0.01$, and significantly different between Pre and Post at $^{**}p < 0.01$ and $^{*}p < 0.05$, respectively.

Before, before experiencing repetitive passive heat loading for 4 weeks; After, after experiencing repetitive passive heat loading for 4 weeks; Pre, before passive heat loading for 30 min; Post, after passive heat loading for 30 min.

Discussion

Meteorological heat waves can endanger human survival. This thermal phenomenon is not just a local or temporary event. Our future may include longer lasting and more serious thermal stress.

However, heat stress due to a number of biological challenges can result in a favorable physiological effect. For example, improved insulin sensitivity is observed after treating patients with type II DM using a constant temperature (41°C) water bath [25].

In our study, we found that repetitive heat loading decreased the levels of insulin and leptin, which are key hormones in the adipoinsular axis, i.e., a bidirectional feedback loop between adipose cell leptin and pancreatic β-cell insulin, and produced favorable effects on whole body sweat loss volume, changes in blood FFA levels, and WS.

Serum insulin level decreased significantly after 4 weeks of PHL. Morera et al. (2012) [26] confirmed that chronic heat loading (35 ± 1°C) in rats leads to improved insulin sensitivity.

Considering that the role of heat shock protein (HSPs) is a natural defense system to prevent insulin resistance [27] and that exposure to hot environments induces HSP expression, as shown Figure 4 [28], the results from this study were hypothesized to be due to increased HSP expression following repetitive PHL, which improved insulin resistance. However, it could also be predicted that insulin production might be suppressed due to activation of the SNS by external heat stimuli.

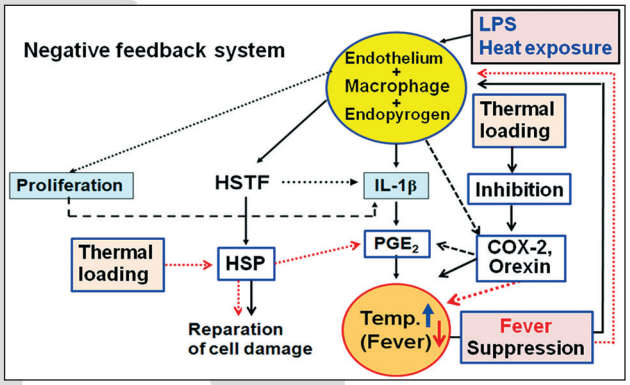

Figure 4. A model of the negative feedback system for thermoregulation with LPS, heat exposure and IL-1β in endothelium, endopyrogen, and/or macrophages. LPS, lipopolysaccharide HSTF, heat shock transcription factor; IL-1β, interleukin-1β; HSP, heat shock protein; PGE2, prostaglandin E2; COX-2, cyclooxygenase-2; Temp., temperature (modified model proposed by Bae et al., 2006) [28].

The suprachiasmatic nucleus (SCN), which has a role as a biological clock, is located in the anterior hypothalamus [29]. The SCN controls glucose production by the liver via sympathetic ganglions projected to paraventricular nucleus and promotes pancreatic insulin production through a parasympathetic ganglion after eating. [30, 31].

In other words, increased sweat production and active sweat gland density by repetitive exposure to thermal stress is a clear effect of SNS activation [14].

Therefore, it is postulated that characteristic short serial SNS activation could affect regulation of the insulin reaction, despite repetitive heat loading sessions, as in our study. This was supported by the significant increase in mean whole body sweat loss volume after PHL.

We conclude that insulin sensitivity improved based on the significant increase in FFA after PHL (Figure 2). Gupte et al. (2009) [13] suggested that fat accumulation can be prevented by weekly heat loading (half body immersion, 41°C, 20 minutes) in high-fat diet fed rats. In other words, heat loading could increase energy demand similar to physical exercise, and lead to a systemic metabolic improvement effect by decreasing FFA accumulation.

Because removing visceral fat in humans can lead to decreased insulin production [2], the reduction in visceral fat through the heat loading test conducted in this study could be a potential factor to lower insulin levels.

The significant decrease in WS after PHL directly indicated a decrease in visceral fat.

As suggested in Figure 1, blood leptin level was very closely related to insulin expression. Therefore, the main cause for the significant decrease in leptin level after repetitive PHL was thought to be the sequential result of repressed insulin expression.

Because leptin expression is upregulated by insulin but downregulated by SNS activation [32], sympathetic activation induced by PHL was also thought to influence the inhibited leptin expression.

Leptin gene (ob) mRNA expression is repressed if the fat decreases. because the feedback signaling loop between adipose cells and the hypothalamus acts to decrease ob gene expression to maintain a constant body weight by reacting to increased energy consumption. The cause of inhibited ob mRNA expression after physical activity is a decrease in insulin level and activation of the SNS [33].

Therefore, the effect of PHL on decreased fat accumulation and the increase in FFA might have directly influenced leptin or the adipoinsular axis reaction indirectly.

We found that repetitive PHL for 4 weeks induced a decrease in insulin and leptin levels, which are major hormones related to the adipoinsular axis. Therefore, we expect a practical heat loading application for its potential physiological benefit not only to decrease fat accumulation but to improve insulin sensitivity.

We hypothesize that HSP expression, particularly HSP72 and HSP25, is a major insulin factor related to the adipoinsular axis reaction, but further experimental study is required to confirm this hypothesis.

Conclusion

We identified the influence of repetitive heat loading (PHL, half bath, 43°C) for 4 weeks (three times/week, 30 minutes/day) on the adipoinsular axis reaction in humans. The results showed significant decreases in blood insulin and leptin levels after repetitive PHL. A reduction in visceral fat and improved insulin sensitivity could be major factors involved in the sequential reaction of the adipoinsular axis.

Abbreviations

PHL: passive heat loading, **SNS**: sympathetic nervous system,

SCN: suprachiasmatic nucleus, **DM**: diabetes mellitus, **FFA**: free fatty acids, **WS**: waist size

HSP: heat shock protein

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Intraventricular clot and possible pathogenic role in cardiac death

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Abstract

The presence of intraventricular clot is a dangerous occurrence that can act on the event death in many ways.

The authors present a case of cardiac death by ventricular hypertrophy and cardiac-respiratory failure.

The autoptic finding of two intraventricular clots suggests a formation subsequent to the hypertrophy and the peculiar position in which have been reported explains a mechanical action on atrioventricular flow rather than on a less likely, although not excluded, arrhythmogenic stimulation.

Key words: Ventricular hypertrophy, clot, cardiac death.

Introduction

The formation or transit of a clot in the intracardiac side is a particularly dangerous.

The reasons of this danger are in pre-existing co-morbidities (1, 2) that usually has necessitated the surgical approach with which this complication is more frequently associated (3-5).

Another reason concerns the problems of diagnosis (2) and differential diagnosis with other pathological forms of intracardiac masses as the myxoma (6).

Also the timely therapeutic solution becomes a determinant variable of success in appropriate management of patient (7) because the most feared risk is the ischemic- infarctual impairment also at the distance from the heart (8). Compared to the outside clot, intrapericardial (5), that acts with an action of extrinsic compression of the cardiac chambers, the intraventricular clot is susceptible of different pathogenic interpretations.

In this manuscript we describe a possible pathogenic mechanism of cardiac death by intraventricular clot.

Case report

A 62-year-old man, the length of 165 cm and weighing approximately 130 kg. History of hypertension and diabetes in pharmacological treatment with enalapril and metformina.

The examination of the thanatological phenomena showed the following findings. Hypostasis in dorsal surface of the body and up to the middle axillary line. Stiffness resolved and present only at legs.

There were no putrefactive phenomena.

In the inspection of the oral cavity there was leakage of serous fluid.

Dissected the soft parts we founded the integrity of the rib cage, diaphragm and bowel loops with the abdominal cavity dry.

Removed the sternal plastron lungs were expanded, without adhesions.

Pericardium were intact and to the opening of which were found few cc of serous fluid.

Heart of globular form (750 g weight, 15 cm longitudinal diameter, 14,5 cm transverse diameter, 4,5 cm anteroposterior diameter).

The thickness of the apical and free wall of the left ventricle were approximately 2 cm. The thickness of the interventricular septum 1,5 cm.

In the cardiac section endocardium shiny appearance without alterations affecting the valve, chordae tendineae or papillary muscles. In the sub-mitral side, in the context of the chordae, was clot with elongated shape and size about 7 cm, yellowish color and soft texture.

To the opening of the right ventricular there was other clot of 5 cm.

To the inspection of the coronary there were not calcification wall and stenotic lumen.

Lungs with increased dimensions and consistency (weight 740 gr right and 840 gr left) and sub-crackling. Large, medium and small bronchi with mucosal hyperemic and occupied by pinkish-

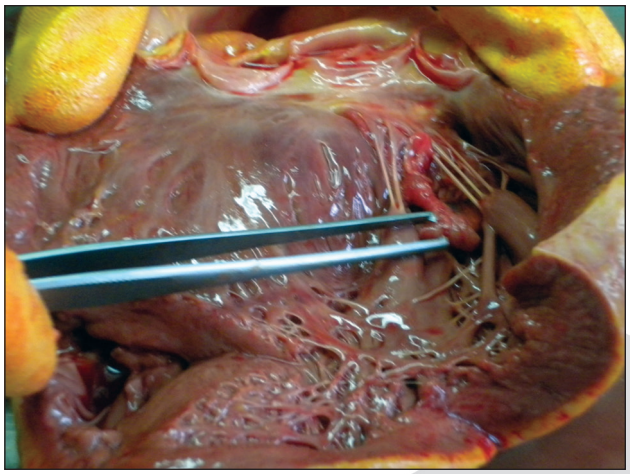

Figure 1. Appearance of the clot to the left ventricular section

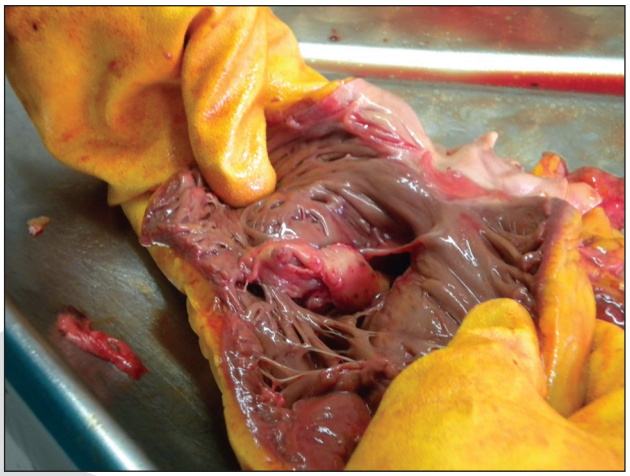

Figure 2. Right ventricular with clot

white foamy liquid. To the full-thickness section of lungs parenchyma was congested, a finding corroborated by squeezing with frothy bleeding.

Discussion

Ventricular hypertrophy, in literature based on hypertensive or valvular pathology (9), is usually associate with thickening of the myocardium and intraventricular volume reduction resulting from the increased systolic blood pressure.

However in this case compared to a considerable increase in the weight of the heart, we see only a modest increase in the thickness of the ventricular wall.

The explanation must be sought in the concurrent need of the ventricles to accommodate a large volume of blood and, therefore, in a simultaneous increase in the diastolic pressure. Also for this reason our case differs from those described in the literature of intra-atrial clot (10), which as site is more sensitive, also for the dynamic ventricular, to the stasis and then to the formation of clot.

The depletion of the functional cardiac pump, fortunately not associated with coronary artery disease, has caused a stasis of blood and, therefore, pulmonary edema and congestion found in autopsy.

However, the process of cardio-respiratory failure has been established in a chronic and presumably not rapid mode, allowing time to clot formation bilaterally.

As described in the literature, patients with cronic heart failure and sinus rhythm have a higher probability of formation of compact plasma fibrin clots and, therefore, of thromboembolic complications (1).

Given the location in which they were found, in the context of the chordae tendineae that have an important function because transmit the contraction and relaxation of the papillary muscles during the cardiac cycle, thus ensuring the closing of the leaflets, contribute to the maintenance of the ventricular architecture and its efficient function in maintaining the cardiac output (11), it is possible easily recognize their contribution to the pathogenic significant impairment of atrioventricular valve function that has probably accelerated in the final phase the cardio-respiratory failure.

But also it is not possible exclude, even if less likely in this case, the potential arrhythmic mechanisms of substances released by dense core granules, alpha-granules, and platelet lysosome (12).

Conclusion

The death of the subject can be attributed to cardio-respiratory failure by ventricular hypertrophy and pulmonary edema, complicated by clotting that has contributed to the functional deficiency of the atrioventricular valve system.

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Butterfly appearance or butterfly effect of silicosis due to denim sandblasting

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Abstract

Background: The occupation of sandblasting denim jeans is relatively new and has developed as a result of changes in fashion in developed countries and the demand for worn-looking jeans. We aimed the role of contrast-enhanced (CE) MRI in the evaluation of progressive massive fibrosis (PMF).

Case: A 48-yr-old male patient had a history of working as a sandblaster for 4 years and had diagnosis of accelerated silicosis due to denim sandblasting for 5 years. Chest X-ray and MRI were taken simultaneously.

Conclusions: In patients with acute and accelerated silicosis, pulmonary artery diameters were shown to be correlated with the aggressiveness of the disease.

Key words: Silicosis, magnetic resonance imaging, PMF.

Introduction

Silicosis is a well-known occupational disease although new occupational causes of silicosis continue to be reported. Progressive massive fibrosis (PMF), which is a characteristic finding of silicosis, is defined as a fibrotic lesion having a diameter of more than 1 cm, and it is classically described as a fibrotic mass surrounded by emphysematous lung fields(1,2). In this study, we aimed to investigate the role of CE-MRI in the evaluation of PMF.

Case Report

A 48-yr-old male patient admitted to the hospital with the complaints of breathlessness, cough and sputum. He had a history of working as a sandblaster for 4 years and had diagnosis of accelerated silicosis due to denim sandblasting for 5 years. Pulmonary function tests showed severe obstructive defect and moderate hypoxia. His chest x-ray and MRI, T2 weighted image, showed solid lesions with irregular border in parahilar area bilaterally. Without and with contrast enhanced T1 weighted MRI images showed parahilar solid lesions and slightly contrast enhancement of the lesions and enlarged pulmonary artery. The findings resembled pulmonary arterial aneurysm or feeding vessel sign (Figure 1).

Discussion

Silicosis is an occupational pulmonary disease known to occur due to the inhalation of silica particles. Silicosis can progress with denim sandblasting and in many other occupations (1). Silicosis has three clinical appearances according to the formation process: acute, accelerated, and chronic. With higher exposures, the disease emerges in a much smaller period of time. For example, in denim sandblasting, the disease can develop in mere months because of dense exposure (acute or accelerated silicosis). In occupations where the exposure rate is lower, or where preventive precautions are taken, it can take much longer to develop (chronic silicosis) (2).

Progressive massive fibrosis (PMF), which is a characteristic finding of silicosis, is defined as a fibrotic lesion having a diameter of more than 1 cm, and it is classically described as a fibrotic mass surrounded by emphysematous lung fields (3). PMF is usually in the upper lung zones but in this case, we have seen bilaterally central zone.

Denim sandblasting is a recent cause of silicosis with fatal outcomes. Because the exposure to silica dust is very high in confined places without any protection, it has clinically and radiologically worse outcomes compared to the other exposures (4, 5).

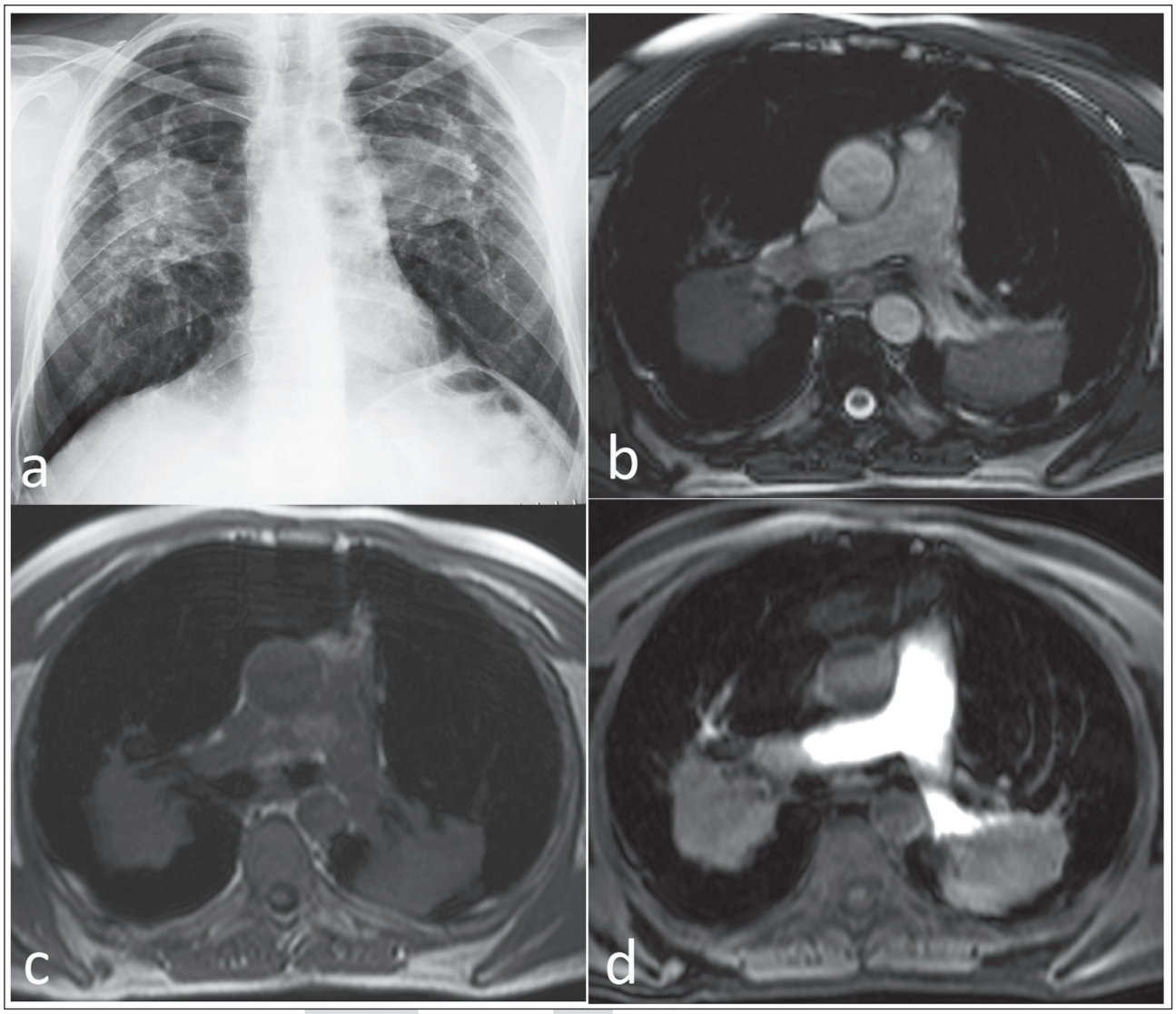

Figure 1. a.) PA Chest X-ray shows parahilar PMF lesions in both lungs. b.) Axial T2 weighted image, c. Axial T1 weighted precontrast image and d. Axial T1 weighted postcontrast image show slightly contrast enhanced solid lesions in both lung parenchyma.

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Different theories on pathogenesis of bisphosphonate-related osteonecrosis of the jaw - A review

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Abstract

Bisphosphonates (BPs) are used to manage osteoporosis, Paget's disease, multiple myeloma, bone cancer and metastases, hypercalcemia of malignancy, fibrous dysplasia and chronic osteomyelitis. BPs reduce pain, pathological fractures, the size of osteolytic lesions and limit metastasis in certain types of cancers. Despite the undoubted benefits of taking bisphosphonates, this therapy carries the risk of side effects like: flu-like symptoms, fatigue, anaemia, gastrointestinal intolerance, atypical femur fractures, oesophageal cancer, atrial fibrillation, chronic musculoskeletal pain, electrolyte imbalance and osteonecrosis of the jaw. Bisphosphonate-related osteonecrosis of the jaw (BRONJ) greatly reduces quality of life and could cause further complications associated with a significant deterioration in the general condition of the patient. It also remains a challenging complication to treat. Pathomechanism of bisphosphonate-related osteonecrosis of the jaw is not fully understood. There are several hypothesis about the mechanisms that lead to the development of this condition. The aim of this paper is to present current concepts on the pathomechanism of bisphosphonate related osteonecrosis of the jaw like cytotoxic effects on osteoclasts, inhibition of angiogenesis, direct cytotoxic effect on oral mucosa, calcification, role of macrophages and genetic risk factors in this pathology. Clarification of the pathomechanism of BRONJ would be the topic issue in improving effects of therapy and minimizing the side effects. Individually chosen therapy with revised doses, exposure time and removal of some risk factors without affecting clinical activity would be the future direction in bisphosphonate treatment.

Key words: Bisphosphonate-related osteonecrosis of the jaw, pathogenesis, bisphosphonates.

Introduction

Bisphosphonates (BPs) are substances used to treat osteoporosis, Paget's disease, multiple myeloma, bone cancer, bone metastases, hypercalcemia of malignancy, fibrous dysplasia, chronic osteomyelitis, osteogenesis imperfecta and gigant cell tumours of the jaw. In these pathological conditions they reduce complications such as pain and pathological fractures. They also reduce size of osteolytic lesions and limit metastasis in certain types of cancers (1). Despite the undoubted benefits of taking bisphosphonates, this therapy carries the risk of side effects (3). The most common are flu-like symptoms, fatigue and anaemia as well as gastrointestinal intolerance (inflammation of the oesophagus, stomach pain, diarrhoea and constipation). The other possible complications include atypical femur fractures, oesophageal cancer, atrial fibrillation and chronic musculoskeletal pain. Patients given BPs intravenously are also at risk of electrolyte imbalance (3). One of the most important complications of bisphosphonates therapy is osteonecrosis of the jaw (BRONJ) which is an area of uncovered bone in the maxillofacial region that has not healed 8 weeks after identification by a health care provider, in a patient receiving treatment or earlier exposed to bisphosphonate therapy without previous radiation therapy to the craniofacial region (4). The most common symptom of BIONJ is the presence of areas of exposed bone, usually painless. Denuded bone has white to yellowish colour with smooth or sharp edges. These changes are sometimes accompanied by paresthesia or neuralgia in the area of the trigeminal nerve innervations. In case of secondary infections symptoms of chronic osteomyelitis appear like jaw and teeth pain, inflammation of the gums,

redness of the mucous membrane, ulcerations, oedema, purulent exudates or presence of fistulas (external or intraoral). In advanced lesions pathologic fractures or osteolysis extending to the interior border of mandible can be found (5). Changes occur most often after 6-18 months of starting treatment with bisphosphonates administered intravenously (6). For oral administration of the drug, the risk of change is greatest after 2-3 years of treatment, or earlier in case of combination therapy with glucocorticoids (7). The incidence of this complication, evaluated in retrospective studies varies, depending on the criteria. The exact incidence of BRONJ is unknown, but reports range from 1% to 11% for patients receiving intravenously administered BPs and 0,1-1% or less for oral BPs (8). It has been proven that bisphosphonate-induced osteonecrosis of the jaw greatly reduce quality of life and could cause further complications associated with a significant deterioration in the general condition of the patient (9). Due to the fact that the management of this complication is problematic - prevention in this case is superior to treatment. Good prevention must be based on the knowledge of the pathogenesis of BRONJ. Multiple factors have been involved in the development of BRONJ but pathomechanism of bisphosphonate-related osteonecrosis of the jaw is not fully understood (10). There are several hypothesis about the mechanisms that lead to the development of this condition. This means that the pathogenesis is complex and requires further investigation.

Material and Methods

The authors performed a literature review in MEDLINE/ PubMed by the main key words related "bisphosphonates" [All Fields]) AND ("osteonecrosis of the jaw") [MeSH Terms] OR "osteonecrosis" [All Fields]) AND ("etiology" [Subheading] OR "etiology" [All Fields] OR "pathogenesis" [All Fields]) AND mechanism [All Fields] AND "risk factors"). Titles and abstracts of the searches were initially screened by two independent reviewers for possible inclusion in the review. Any disagreement was resolved by discussion. Manual search of the bibliographies of all full-text articles and related reviews, selected from the electronic search, were also performed. The cited literature was evaluated and a traditional manual search and library research was additionally performed. In this paper we are going to review different theories on pathogenesis of bisphosphonate-related osteonecrosis of the jaw.

Mechanism of action of BPs

Bisphosphonates are analogues of pyrophosphate that have high affinity for hydroxyapatite and the ability to bind to sites with active bone metabolism. Because of their substitution of carbon for oxygen in the molecule, bisphosphonates are resistant to hydrolytic breakdown. These substances can be divided into three generations. The first generation are non-nitrogen containing BPs (etidronate, clodronate, tiludronate), mainly used for treating Paget's disease. In the second generation, the molecule is substituted with nitrogen (pamidronate, alendronate, ibandronate). The third generation BPs feature nitrogen-substituted ring-structures (risedronate, zoledronate). The relative potency of the drug increases with nitrogen substitution and even more so with the integration of a ring structure (8). Non-nitrogen containing BPs are taken up by the osteoclast and antagonise the cellular energy pathways leading to cell apoptosis. This group is metabolised to non-hydrolyzable cytotoxic ATP analoges that inhibit ATP-dependent enzymes. Nitrogen containing bisphosphonates have a more complex pathway of action by inhibiting the farnesyl diphosphate synthase – a key enzyme of the mevalonate pathway, which affects the osteoclastogenesis, apoptosis and cytoskeletal organization and cell-cycle dynamics. The osteoclasts lose their normal rippled border at the Howship lacuna resorption site and die (11). After the administration of BPs, bone remodelling is jeopardized but bone tissue continues to mineralize in a uniform way, collagen becomes more highly cross-linked and bone tissue has higher amounts of microdamage (12,13). In consequence, bone becomes fragile, brittle and less elastic (2,5). While healthy bone is constantly subject to microscopic damage, bisphosphonate-induced suppression of remodelling favours the accumulation of microscopic damage in bone. The quantity of accumulated microdamage varies from site to site; it is more pronounced in trabecular than in cortical bone (13).

Pathomechanism of bisphosphonate-related osteonecrosis of the jaw

The first theory focuses on the cytotoxic effect of bisphosphonates on osteoclasts, which results in the inhibition of bone remodelling process. BPs accumulate in the bones and in the consequence of minor trauma (like poorly fitting dentures, caries and periodontal diseases), are released into the environment. Mucosal cells (epithelial cells, fibroblasts, lymphocytes) are stimulated by BPs to produce different cytokines by inhibiting HMGR (hydroxymethylglutaryl coenzyme A reductase, HMG-CoA reductase). HMGR is a enzyme which controls the mevalonate pathway, the metabolic pathway that produces cholesterol and other isoprenoids. Bisphosphonates block HMG-CoA reductase and thereby inhibit osteoclasts function and osteoclasts development from monocytes (14). In the mucosa of patients with BRONJ, HMGR expression is decreased. This could be responsible for the stimulation of mucosal cells to produce IL-6, which stimulates osteoclasts activity. IL-6 induced osteoclasts activity leads to the increase of RANKL (Receptor Activator of Nuclear Factor Kappa-B Ligand) and RANKL/OPG (OPG - Osteoprotegrin) ratio. Moreover, increased IL-6, induced by pro-inflammatory stimuli, is also accompanied by the lowering of osteoblastic differentiation markers (15,16). In the oral mucosa from patients with BRONJ the expression of RANKL, IL-6 and the RANKL/OPG ratio is much higher while HMGR and OPG expression is decreased compared to patients without BRONJ. This effect is stronger with nitrogen-containing bisphosphonates (17). Osteoclasts are derived from macrophages after RANK (Receptor Activator of Nuclear Factor Kappa-B) receptor activation with RANKL produced by osteoblasts. RANKL production is inhibited by naturally occurring cytokine – osteoprotegrin (7). Bisphosphonates inhibit the secretion of osteoprotegerin and stimulate apoptosis of osteoclasts, thereby reduce bone turnover (18).

Another theory highlights BPs' induced inhibition of angiogenesis. VEGF (Vascular Endothelial Growth Factor) modulates angiogenesis and vasculogenesis. It also regulates endochondral ossification. Normal angiogenesis is crucial to tissue repair and osteogenesis during bone repair. BPs lower the level of VEGF in oral mucosa leading to disturbances in the bone synthesis and healing process (19,20). This in consequences causes avascular bone necrosis and cell apoptosis (17,21,22). In the angiogenesis in the jaw a critical role is also played by osteoclasts. At different anatomic sites the contribution of osteoclasts and osteoblasts to angiogenesis differs and osteoblasts have a much more important role in long bones than in the jaw. Even if the angiogenic activity of osteoclasts is inhibited, the long bones could survive by a contribution of osteoblasts to angiogenesis. Bisphosphonate-related osteonecrosis therefore only happens in the jaw (23). Polymorphisms expression of VEGF was investigated by Arduino et al (14). He suggested a possible haplotype effect in BRONJ but results were inconclusive.

Another hypothesis suggest that BPs have a direct toxic action on the oral mucosa. The release of BPs from the jaw bone following local trauma causes epithelial cells damage similar to the pathologies of esophagus connected with oral BPs administration. It is suggested that these substances inhibit the *farnesyl diphosphate synthase*. This leads to alternations of the perilesional mucosa observed by Lorenzo et al (24) – swollen cells, hypereosinophilia, pyknosis without the typical connective tissue inflammation. Due to the periodontal tissues anatomy there is contact between BPs in bones and soft tissues even without traumatic events thus justifying the onset of BRONJ without dental extractions. As a result, oral pathogens are able to pass through damaged oral mucosa and infect the bone, leading to its necrosis (10).

Next theory was presented by Meiller et al (25). They showed the presence of calcium in the connective tissue and blood vessels in patients with bisphosphonate-related osteonecrosis. This process is called calcification and involves the deposition of calcium in the blood and thus causing ischemia, necrosis and difficulties in healing (2,7). Injury, inflammation and infection form an acidic environment, which promotes the release of the bisphosphonate and the secondary release of calcium from the bone (10). The *Actinomyces* species, commonly found on these lesions, can release acids favouring chronic inflammation. After that calcium is deposited in the vessel and perivascular tissue resulting in ischemia of the mucosa, necrosis and mucosal dehiscence with bone exposure

occur. Therefore, it is proposed to neutralise the pH in the oral cavity, restore the calcium-phosphate balance, avoid mechanical injury and reduce the number of oral bacteria (25). According to this theory, initially, there is only a mucosal necrosis and which later develops into osteonecrosis.

A different theory based on the histological examination of necrotic bone was introduced by Marx and Tursun (26). They explained this phenomenon according to the role of osteoclasts. These cells are responsible not only for bone resorption, but indirectly for the differentiation of osteoblasts by the secretion of IGF-1, IGF-2 (Insulin-like Growth Factor 1, 2) and bone morphogenetic proteins (BMPs). This means that the loss of osteoclast activity (BPs induce osteoclast apoptosis) is associated with a progressive decrease in the number of osteoblasts. The periosteal osteoblasts have the ability to renew themselves in a germ layer of the periosteum regardless of osteoclast function and therefore are resistant to the action of bisphosphonates. In the initial stage there is bone necrosis while the periosteum and mucosa remain unchanged. This situation does not last long, because intraosseous blood vessels are a source of nutrition for periosteum. After the development of necrosis blood flow decreases in the bone, resulting in the periosteum ischemia, mucosal necrosis and dehiscence with the exposure of the underlying bone. Bisphosphonates also reduce the number of p63 – positive basal epithelial progenitor cells. p63 is a selective marker of basal stem cells of stratified epithelium, required for initiation of epithelial stratification. Down-regulation of this protein results in delayed wound healing (27).

Another possible explanation of the pathogenesis of BRONJ emphasizes the role of macrophages in this phenomenon. After osteoclasts, monocytes and macrophages are the cells most likely to be affected by the administration of bisphosphonates because of similarities between those groups arising from a common origin of the monocyte-macrophage cell line. Bisphosphonates do not affect neutrophils so the risk of systemic infection is minimal. However, this substances can cause severe defects in the second line of defense. The reduced function and presence of monocytes and macrophages could become critical for the development of BRONJ. The role of macrophages could also explain similar side effect of denosumab and sunitinib (28).

The exclusivity of BRONJ in the jawbone can be explained with Msx-1 - a osteoproliferative transcription factor which induces proliferation and inhibits terminal differentiation of osteoblasts. It plays a fundamental role in tooth development and alveolar bone regeneration by the modulation of bone morphogenetic protein-2 (BMP-2)-related bone homeostasis. Msx-1 is expressed constantly in the periodontal ligament, whereas in long bones is only transiently reactivated during fracture repair. Msx-1 is co- expressed with RANKL and in BRONJ-affected bone expression of both is reduced and the expression of BMP-2 is elevated. This results in suppressed bone remodelling (29,30). Further arguments pointing to the uniqueness of the jawbone compared to other bones are the presence of teeth, which may directly affect the jaw. Inflammation can readily spread into the jawbones by caries, periapical lesions, periodontitis and during invasive dental procedures. Increased blood supply and a high bone turnover compared to other long bones results in increased concentration of bisphosphonates. Adherence of oral bacteria to BP-containing bone is increased compared to control healthy bone. Jawbones are covered with thin mucosal and periosteal membranes that make them vulnerable (1). Necrotic areas twice as likely to occur in the mandible than in maxilla (5). This is due to the fact that the lower jaw has high remodelling rate related to daily activity (repeated mastication) and anatomy (thicker and dense cortical bone and richer bone marrow than maxillary bone), which leads to greater accumulation of bisphosphonates (4). Mandible also has more areas with thin mucosa than the upper jaw (1). Areas of most frequent occurrence of necrotic bone are: mandibular torus, mylohyoid line, palatal torus and exostosis (5,31). The molar region is the most common site of osteonecrosis (4). Changes often occur in areas where extraction was previously performed or other invasive dental treatment like periodontal surgery or implant placement during or after treatment with bisphosphonates (1,5). In addition, changes may occur due to the irritation of the mucous membranes of poorly fitting dentures (1,7). It is also estimated that 40% - 50% of the lesions formed spontaneously without previous trauma (32).

The risk factors for BRONJ

Not all BPs users develop BRONJ. This suggests that environmental and/or genetic differences between patients may affect the development of this complication (33).The potential risk factors for BRONJ are multiple and have not yet been completely identified (1). Bisphosphonates induced osteonecrosis of the jaw most often occurs in connection with the use of the strongest acting drug of the group - *zoledronic* acid (10). Generally nitrogen containing BPs (zoledronic acid, alendronate, risedronate, and pamidronate) show a strong relationship with BRONJ because of their higher affinity for hydroxyapatite and a stronger bone resorption inhibitory action. In the case of the use of oral bisphosphonates, changes are less frequent and less severe (33), possibly due to the low absorption of an oral formulation. In addition, BRONJ is seven times more frequently observed in patients with periodontal disease and the presence of dental infection before starting the therapy, which highlights the need to dental checks prior to the initiation of treatment with bisphosphonates. The risk of developing osteonecrosis is higher for Caucasians, which also increases with age, especially for patients treated with intravenous bisphosphonates for multiple myeloma (5). Additional risk factors, though not unequivocally proven relationships are: steroid therapy, diabetes, smoking, treatment with chemotherapy, hypothyroidism, obesity (1). The combination of BPs and antiangiogenic drugs induces BRONJ more frequently than BPs alone (16% vs. 1.1%) (34). Cyclophosphamide, erythropoietin, bortezomib, glucocorticoids and thalidomide are also listed as risk factors. Some drugs may additively or synergistically impair jaw vascularisation and cause a predisposition to bone necrosis. For example, docetaxel has anti-angiogenic properties and induces leucopoenia and in connection with zoledronic acid multiplies the risk of BRONJ development (35). Other drugs used to treat cancers like bevacizumab, sunitinib and denosumab have a common side effects on the bone presented as osteonecrosis of the jaw, even when used without bisphosphonates (36). Diz et al (37) suggested that there could be a susceptibility factor prevalent in the population of a well-defined geographical region. This hypothesis is based on review of literature which showed that 55% of reports came from Mediterranean countries such as Italy, Israel, Spain and France. The finding does not appear to be conditioned by publication bias.

The possibility to predict which patients may develop BRONJ would help plan the most effective and safe therapy. The exact mechanisms by which genetic variations make some individuals susceptible to BRONJ are not fully understood. Very limited data is available on genetic markers of BRONJ. Nucleotide polymorphisms in CYP2C8, COLIA 1, RANK, MMP2, OPG, OPN, VEGF and RBMS3 genes have recently been reported as possible genetic predisposing factors (6,15).

Cytochrome P450-2C (CYP2C8) is involved in arachidonic acid metabolism and cholesterol biosynthesis and modulates angiogenesis and osteoblast differentiation in bone (33). CYP2C8 participates in the metabolism of many drugs but BPs are not metabolized by P450 enzymes. Despite this fact, CYP2C8 gene regulates other biological processes and modifies osteoblastic and osteoclastic functions by the metabolic cascade for cholesterol synthesis. The passable role of cytochrome P450 CYP2C8 gene has been investigated, although the results are contradictory. Balla et al (38) stated that CYP2C8 rs1934951 polymorphism has an influence on the anatomic localization of BRONJ. In his study the CYP2C8 AG genotype was associated with a 19.2-fold increased risk for mandibular BRONJ compared to the GG genotype. COL1A1, RANK, MMP2, OPG and OPN are the other genes which might play a role in the pathogenesis of BRONJ because their mutations result in the development of osteoporosis, osteoclastogenesis, bone abnormalities, impairment of host defence and wound healing. COL1A1 gene is for the type I collagen, an important protein of bone. Mutation in this gene produces genetic bone disorder – osteogenesis imperfecta (40). RANK and OPG are members of the tumor necrosis factor receptor super family that plays a central role in osteoclast development. RANK plays a central role in osteoclast development and activation of bone resorption. OPG (Osteoprotegerin) is a regulator of bone remodelling. OPN (Osteopontin) is adhesive glycophosphoprotein involved in bone metabolism, angiogenesis, immune system regulation and is essential for osteoclast function. Katz et al (41) observed that patients suffering from multiple

myeloma with single nucleotide polymorphism for COL1A1 rs1800012, RANK rs12458117, MMP2 rs243865, OPN rs11730582 and OPG rs2073618 were more prone to developing BRONJ.

Mutations in gene encoding MMP2 (Matrix Metalloproteinase 2) leads to bone abnormalities like Winchester syndrome, Torg syndrome (multicentric osteolysis) and nodulosis-arthropathy-osteolysis syndrome (NAOsyndrome). Lehrer et al. suggested similarities between those conditions and BRONJ. Another reasons for the studies in this area is that MMP2 is the only gene known to be associated with bone abnormalities and atrial fibrillation – one of the side effects of BPs (6).

RBMS3 is encoding putative RNA-binding protein 3 a binding protein for Prx1 (Paired Mesoderm Homeobox Protein 1), a factor up regulating synthesis of collagen type I, the main part of the bone matrix. BPs also down regulate collagen type I synthesis so single nucleotide polymorphism on RBMS3 (rs17024608) increase the toxic effect of BPs (40).

Conclusion

Despite a growing body of literature, many aspects of BRONJ are still to be clarified. Most favoured theories include the cytotoxic effects on osteoclast and the inhibition of angiogenesis, however these mechanisms do not elucidate all the aspects of pathogenesis of bisphosphonate related osteonecrosis of the jaw. Fully clarification of the pathomechanism of BRONJ would be the topic issue in improving effects of therapy and minimizing the side effects. An interdisciplinary approach involving dentists, medical oncologists, oral and maxillofacial surgeons is required to prevent and manage this condition. Individually chosen therapy with revised doses, exposure time and removal of some risk factors without affecting clinical activity would be the future direction in bisphosphonate treatment. It would be interesting to examine before the onset of therapy, which patients are more prone to BRONJ according to possible genetic risk factors. Hence there is a need for further research in this area.

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Abstract

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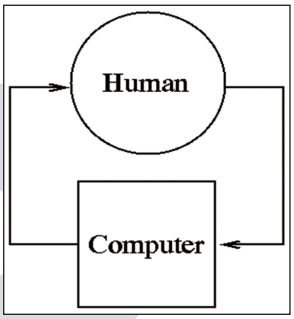

Figure 1. Text here

Conclusion

Be brief and give most important conclusion from your paper. Do not use equations and figures here.

Acknowledgements (If any)

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