Volume 7 / Number 9 / 2013 ISSN 1840-2291

HealthMED

Journal of Society for development in new net environment in B&H

design by Mirza Bastc

Volume 7 / Number 9 / 2013

HealthMED

Journal of Society for development in new net environment in B&H

Address Hamdije Kresevljakovica 7A,
71 000 Sarajevo,
Bosnia and Herzegovina.
Editorial Board healthmedjournal@gmail.com
http://www.healthmed.ba
Published by DRUNPP, Sarajevo
Volume 7 Number 9, 2013
ISSN 1840-2291
e-ISSN 1986-8103

HealthMED journal is indexed in:

- EBSCO Academic Search Complete
- EBSCO Academic Search Premier,
- EMBASE,
- SJR Scopus,
- Index Copernicus,
- Science Citation Information Expanded (SCIE),
- Electronic Social and Science Citation Index (ESSCI),
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Evaluation of dose efficacy of treatment with atorvastatin, rosuvastatin and simvastatin in patients with hyperlipidemia

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Abstract

Objective: Atherosclerotic heart diseases continue to be the most important cause of death in developed countries. Hyperlipidemia is the most crucial risk factor in atherosclerosis development. The statins used in hyperlipidemia treatment play an essential role in reducing the morbidity and mortality associated with atherosclerosis. There are numerous studies on old generation statins and new generation statins; however, there are limited number of studies comparing these two groups. Therefore, we aimed to present this study to the literature and determine the dose efficacy of hypolipidemic agents by comparing the old generation statin simvastatin to new generation statins (atorvastatin, rosuvastatin).

Methods: This study is a clinical, prospective cohort study. A total of 160 subjects (76 women and 84 men) who applied to our clinic from November 2011 to May 2011 and were indicated for medicinal treatment according to National Cholesterol Education Programme Adult Treatment Panel (NCEP ATP) 3 criteria despite the four week long first line diet were included in the study. Following the evaluation of lipid profiles based on medical history, physical examination, and clinical and laboratory findings, eligible subjects were assigned to the three groups according to simvastatin dose (10 mg, 20 mg, 40 mg/day), three groups according to atorvastatin dose (10 mg, 20 mg, 40 mg/day) two groups according to rosuvastatin dose (10 mg, 20 mg/day). Thus, a total of 8 groups were generated. There were 20 patients in each group. In our study, the subjects were evaluated with clinical and laboratory methods at baseline and after 6 weeks of treatment.

Results: The mean age of the 160 patients enrolled for the study was 58.95±10.22 (37 to 82). There was no difference between the groups with regards to demographic characteristics. The reduction in low-density lipoprotein (LDL) cholesterol was 28-40% (10-40 mg/day) with simvastatin, 39-51% (10-40 mg/day) with atorvastatin and 50-60% (10-20 mg/day) with rosuvastatin after 6 weeks of treatment ($p<0.01$). The increase in high-density lipoprotein (HDL) cholesterol at week 6 compared to baseline was most prominent in the rosuvastatin (20 mg/day) group.

Conclusion: We detected that the hypolipidemic effects of rosuvastatin and atorvastatin were more prominent compared to simvastatin. This supports the idea that new generation statins may be used in clinical practice to a further extent compared to old generation statins. Furthermore, based on the findings of our study it can be concluded that rosuvastatin may be the preferred choice of treatment in hyperlipidemia patients with low levels of HDL-C.

Key words: Hyperlipidemia, statins, dose efficacy.

Introduction

Cardiovascular diseases are the most important cause of death in developed countries[1]. Coronary heart disease (CHD) is the leading cause of death. Atherosclerosis is a complicated inflammatory and fibroproliferative response leading to atherogenic lipoprotein aggregation in arterial intima, and it plays a major role in CHD development. Hyperlipidemia is an important risk factor in CHD development, and can be reduced with cholesterol lowering treatment. Studies have shown decreased cardiovascular mortality and morbidity with reduced levels of LDL-C[2-4]. Therefore, statins with LDL-C lowering and pleiotropic effects have come into prominence[5-8]. Numerous studies have shown that statins, which are HMG CoA reductase

inhibitors reduce the levels of LDL-C by 28% to 55% on average.

There are numerous studies on old generation statins and new generation statins; however, there are limited number of studies comparing these two groups. Therefore, we aimed to present this study to the literature and determine the dose efficacy of hypolipidemic agents by comparing the old generation statin simvastatin to new generation statins (atorvastatin, rosuvastatin).

Material and Methods

Patient population

A total of 160 subjects (76 women, 84 men) who applied to cardiology policlinics from November 2011 to May 2011 were included in the study. Subjects received a first line diet for four weeks.

Inclusion criteria

Subjects who had low risk according to NCEP-ATP III with LDL-C at and above 190 mg/dL those who had intermediate risk with LDL at and above 160 mg/dL and those who had intermediate-high risk with LDL-C at and above 160 mg/dL following the 4 week long first line diet, patients with clinical evidence of atherosclerosis, and subjects with equivalent of coronary heart disease (peripheral artery disease, abdominal aortic aneurysm, symptomatic carotid artery disease, diabetes mellitus, risk of coronary artery disease in 10 years >20%) were included in the study[9].

Exclusion criteria

Subjects with the following were excluded from the study: known hypersensitivity to HMG-CoA reductase inhibitors, fasting triglyceride levels >400 mg/dL, use of lipid lowering agents within the last two months, pregnancy or breast-feeding, uncontrolled hypothyroidism, nephrotic syndrome, severe renal insufficiency, alcohol abuse, secondary hyperlipidemia reasons such as hepatobiliary diseases, known muscle disease, malignancy or any other serious medical condition, use of drugs such as cyclosporine, erythromycin, itraconazole, antihistamine, fibrate which are known to increase the risk of rhabdomyolysis in concomitant use with HMG-CoA reductase inhibitors.

Study Design

This study is a clinical, prospective cohort study. Following the evaluation of lipid profiles based on medical history, physical examination, and clinical and laboratory findings, eligible subjects were assigned to the three groups according to simvastatin dose (10 mg, 20 mg, 40 mg/day), three groups according to atorvastatin dose (10 mg, 20 mg, 40 mg/day) two groups according to rosuvastatin dose (10 mg, 20 mg/day). Thus, a total of 8 groups were generated. There were 20 patients in each group. In our study, the subjects were evaluated with clinical and laboratory methods at baseline and after 6 weeks of treatment.

Laboratory assessment

Lipid profiles were measured by means of the spectrophotometric method using autoanalyzer in biochemistry laboratory.

Statistical Analysis

Data were presented as numbers, percentage, mean and standard deviation. Data analysis was performed using the SPSS 18 computer program. Wilcoxon analysis was used for the comparison of lipid profiles before and after the treatment with simvastatin, atorvastatin and rosuvastatin.

Results

The study was conducted in a total of 160 subjects from November 2010 to May 2011. The age of subjects varied from 37 to 82 with an average of 58.95±10.22. 76 (47.5%) of the subjects were female and 84 (52.5%) were male. Demographic characteristics are shown in Table 1. Smoking was present in 25.6% of the subjects while 17.5% had cardiovascular disease in their families, 25.6% had CHD, and 21.9% had diabetes (DM). There was no significant difference between the groups with regards to demographic characteristics. Smoking rate was significantly higher in men compared to women.

The reduction in total cholesterol levels (Table 2), the reduction in LDL-C levels (Table 3) and the reduction in triglyceride (TG) levels (Table 4) at week 6 compared to baseline was statistically significant in all statin groups ($p<0.01$ in all groups).

Table 1. Demographic characteristics of the subjects

	n	Percent (%)
Gender		
Female	76	47.5
Male	84	52.5
Smoking	41	25.6
DM	35	21.9
HT	67	41.9
Family history	28	17.5
CHD	41	25.6

Abbreviations; DM: Diabetes mellitus, HT: Hypertension, CHD: Coronary Heart Disease

The increase in HDL-C levels at week 6 compared to baseline was most prominent in the rosuvastatin 20 mg/day dose group (Table 5).

Discussion

Currently, atherosclerosis is the most important cause of mortality, particularly in developed countries[10]. HT, smoking, hyperlipidemia and DM are the most important modifiable risk factors of atherosclerosis[10-14]. In terms of hyperlipidemia; high levels of total cholesterol, LDL-C and triglycerides,

Table 2. Comparisons of total cholesterol levels

	Number of Patients	Pre-treatment AV and SD	Post-treatment BS and SD	P value	Change %
Simvastatin 10 mg	20	223.05±31.20	176.90±25.04	<0.01	20
Simvastatin 20 mg	20	240.25±42.02	180.75±32.01	<0.01	24
Simvastatin 40 mg	20	232.65±28.36	166.50±23.47	<0.01	28
Atorvastatin 10 mg	20	239.25±49.32	172.05±35.65	<0.01	28
Atorvastatin 20 mg	20	231.30±38.18	148.50±27.46	<0.01	35
Atorvastatin 40 mg	20	238.70±39.56	147.90±27.03	<0.01	38
Rosuvastatin 10 mg	20	226.45±46.70	141.75±22.72	<0.01	37
Rosuvastatin 20 mg	20	252.75±32.20	138.85±16.80	<0.01	44

AV: Average value, SD: Standard deviation

Table 3. Comparisons of LDL cholesterol levels

	Number of Patients	Pre-treatment AV and SD	Post-treatment AV and SD	P value	Change %
Simvastatin 10 mg	20	153.10±28.83	109.20±19.52	<0.01	28.7
Simvastatin 20 mg	20	168.35±28.72	111.85±22.15	<0.01	33.5
Simvastatin 40 mg	20	164.30±22.99	98.20±13.73	<0.01	40.2
Atorvastatin 10 mg	20	169.85±40.85	103.40±29.26	<0.01	39.1
Atorvastatin 20 mg	20	160.10±32.53	83.00±20.45	<0.01	48.1
Atorvastatin 40 mg	20	169.40±30.17	82.90±18.61	<0.01	51.1
Rosuvastatin 10 mg	20	159.90±34.53	79.80±16.44	<0.01	50.1
Rosuvastatin 20 mg	20	182.25±28.67	71.85±15.79	<0.01	60.5

AV: Average value, SD: Standard deviation

Table 4. Comparisons of triglyceride levels

	Number of Patients	Pre-treatment AV and SD	Post-treatment AV and SD	P value	Change %
Simvastatin 10 mg	20	177.15±65.21	150.90±48.60	<0.01	14.8
Simvastatin 20 mg	20	185.95±67.69	149.45±50.40	<0.01	19.6
Simvastatin 40 mg	20	188.05±42.33	158.35±36.29	<0.01	15.7
Atorvastatin 10 mg	20	176.25±59.61	144.80±53.31	<0.01	17.8
Atorvastatin 20 mg	20	164.50±57.44	125.05±43.17	<0.01	23.9
Atorvastatin 40 mg	20	179.90±60.70	124.80±42.23	<0.01	30.6
Rosuvastatin 10 mg	20	175.65±56.92	132.50±44.04	<0.01	24.5
Rosuvastatin 20 mg	20	188.30±55.53	143.70±35.87	<0.01	23.7

AV: Average value, SD: Standard deviation

Table 5. Comparisons of HDL cholesterol levels

	Number of Patients	Pre-treatment AV and SD	Post-treatment AV and SD	P value	Change %
Simvastatin 10 mg	20	43.40±10.39	45.20±9.80	0.003	4.1
Simvastatin 20 mg	20	41.65±9.46	44.10±8.26	0.013	5.8
Simvastatin 40 mg	20	39.40±7.59	41.35±7.14	0.008	4.9
Atorvastatin 10 mg	20	43.05±13.41	45.45±12.86	0.005	5.5
Atorvastatin 20 mg	20	41.70±8.57	43.40±9.60	0.10	4.1
Atorvastatin 40 mg	20	42.55±11.98	43.15±12.80	0.87	1.4
Rosuvastatin 10 mg	20	38.40±6.87	40.10±7.16	0.07	4.4
Rosuvastatin 20 mg	20	42.55±8.08	46.70±9.26	0.003	9.7

AV: Average value, SD: Standard deviation

and low levels of HDL-C are known to increase the risk of atherosclerosis. Particularly the LDL-C level shows a linear association with CHD development. This association renders LDL-C as the primary target in hyperlipidemia treatment. The studies on first generation statins targeting LDL-C (simvastatin, pravastatin and fluvastatin) have shown the safety and efficacy of these agents. In the subsequent years, numerous studies were performed with the more efficient agents, atorvastatin and rosuvastatin, and these drugs have been introduced to clinical practice.

Dose efficacy of simvastatin, atorvastatin and rosuvastatin

One of these studies, the STELLAR (Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses) study, including 2431 patients investigated the effects of rosuvastatin vs. atorvastatin, simvastatin and pravastatin on LDL-C and other lipids in patients with hypercholesterolemia[15]. In conclusion, rosuvastatin decreased LDL-C levels by 8% more compared to atorvastatin, 26% more compared to pravastatin, and 18% more compared to simvastatin. Rosuvastatin and atorvastatin also provided more reduction in TG levels compared to other statins. While the favorable effects of rosuvastatin, simvastatin and pravastatin on HDL-C become more significant with dose escalation, the HDL-C increasing effect of atorvastatin significantly decreases with dose escalation. The decrease in HDL-C increasing effect of atorvastatin with dose escalation has been shown in other studies; and the mechanism remains unclear. Consistent with the STELLAR study, our study has investigated the effect of six week long statin treatment on serum lipid profile in patients with hyperlipidemia. Our study has allowed us to compare the old generation simvastatin against the new generation atorvastatin and rosuvastatin. The known hypolipidemic effects of statins on total cholesterol, LDL-C and TG levels were also observed in our study, and significant decreases were found between the pre- and post-treatment parameters. As expected, a mild increase was seen in HDL-C levels.

Similarly, in the CURVES (Comparative Dose Efficacy Study of Atorvastatin Versus Simvastatin, Pravastatin, Lovastatin, and Fluvastatin in Patients With Hypercholesterolemia) study including 534 patients the effect of atorvastatin, simvastatin, pravastatin, lovastatin and fluvastatin were investigated following 8 weeks of treatment[16]. Atorvastatin was found to decrease the LDL-C, TG and total cholesterol levels more effectively compared to the other statins in this study. However, this study showed a decrease in the HDL-C increasing effect of atorvastatin with dose escalation.

The Scandinavian Simvastatin Survival Study (4S) study was the first among extensive, prospective and long term study comparing the old generation statin, simvastatin against placebo. This study was conducted in 4444 CHD patients with hyperlipidemia. Patients were compared with the placebo group after treatment with 10-40 mg simvastatin following 8 weeks of diet, and the follow-up duration was 5.4 years. This study showed a 25% reduction in total cholesterol levels, 35% reduction in LDL-C levels and 10% reduction in TG levels while total mortality decreased by 29%, and cardiac mortality decreased by 42%[2]. This study is important as it clearly demonstrates the benefits of secondary prevention in CHD.

1036 subjects with LDL-C levels above 160 mg/dL were included in the ECLIPSE (Efficacy and tolerability of rosuvastatin and atorvastatin when force-titrated in patients with primary hypercholesterolemia) study on new generation statins and similarly, rosuvastatin treatment administered at the same dose with atorvastatin was shown to be more effective at the end of 6 weeks[17].

The ARIES (Comparison of efficacy and safety of rosuvastatin versus atorvastatin in African-American patients in a six-week trial) study on new generation statins included 774 subjects and compared the lipid profiles following a 6 week treatment with rosuvastatin (10, 20 mg/ day) and atorvastatin (10, 20 mg/ day). The reductions in atorvastatin arm (in 10 mg and 20 mg groups) were 32% and 38% in LDL-C levels, 17% and 20% in TG levels, and 23% and 28% in total cholesterol levels, respectively. The HDL-C levels were increased by 5% in both dose groups. The reductions in rosuvastatin arm (in 10 mg and 20 mg groups) were 37% and 45% in LDL-C levels, 16% and 21% in TG levels, and 27% and 33% in total cholesterol levels, respectively. Rosuvastatin was found to be 2-3% more effective on HDL-C compared to atorvastain[18].

Our study is important as it compares the new generation statins, atorvastatin and rosuvastatin with the old generation statin, simvastatin in hyperlipidemic subjects. The efficacy of 6 week long statin treatments at different doses were evaluated in our study. In conclusion, reductions were detected in LDL-C values (28%, 33%, 40%, respectively), total cholesterol values (20%, 24%, 28%, respectively) and TG levels (14%, 19%, 15%, respectively) in the simvastatin groups (10, 20, 40 mg/day). However, HDL-C levels were shown to be increased (4%, 5%, 5%, respectively). Reductions were detected in LDL-C values (39%, 48%, 51%, respectively), total cholesterol values (28%, 35%, 38%, respectively) and TG levels (14%, 19%, 15%, respectively) in the atorvastatin groups (10, 20, 40 mg/day) while HDL-C levels were found to be increased (4%, 5%, 5%, respectively). Reductions were detected in LDL-C values (50%, 60%, respectively), total cholesterol values (37%, 44%, respectively) and triglyceride levels (24%, 23%, respectively) in the rosuvastatin groups (10, 20, mg/day). However, HDL-C levels were shown to be increased (4%, 9%, respectively). In light of the present findings, rosuvastatin was found to be the most effective statin in our study. Rosuvastatin was found to be the most effective statin. It was also found that rosuvastatin had a more rapid rate of increase regarding the HDL-C levels with dose escalation compared to that of atorvastatin. Furthermore, all three statin treatments were found to be dose-effective and reliable.

Dose safety of simvastatin, atorvastatin and rosuvastatin

In our study, no side effects were observed in patients receiving simvastatin while one patient receiving rosuvastatin 10 mg/day and one patient receiving atorvastatin 40 mg/day had 3-fold increases in aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels. The liver function tests were normalized following cessation of treatment. In a patient receiving rosuvastatin 20 mg/day had elevated levels of creatinine kinase (CK). The patient was taken into follow-up following cessation of treatment and CK levels were normalized. One patient receiving atorvastatin 20 mg/day and another patient receiving 40 mg/day were taken into follow-up due to myalgia complaint without elevated CK levels. Their complaints were resolved upon discontinuation of treatment. It is important to consider myopathy and hepatotoxicity during the use of statins in clinical practice.

Limitations of the present study include the limited number of patients and the lack of evaluation regarding long term morbidity and mortality.

Conclusion

Statins are the most effective and reliable agents to be used to achieve the target values of total cholesterol and LDL-C. While the favorable effect of rosuvastatin treatment on LDL-C was found to be more prominent in our study, the findings were consistent with the previous studies. We detected that the hypolipidemic effects of rosuvastatin and atorvastatin were more prominent compared to simvastatin. This supports the idea that new generation statins may be used in clinical practice to a further extent compared to old generation statins. Furthermore, based on the findings of our study, it can be concluded that rosuvastatin may be the preferred choice of treatment in hyperlipidemia patients with low levels of HDL-C.

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α_2-antiplasmin and plasminogen, and the risk of myocardial infarction in women

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Abstract

Investigations about the relationship between Plasma levels of fibrinolytic proteins and myocardial infarction have been conflicting. As measured by a plasma-based assay, decreased fibrinolytic potential has been shown to be a risk factor for arterial thrombosis. The Study of Myocardial Infarctions Leiden showed that increased levels of α_2-antiplasmin are associated with an increased risk of myocardial infarction in men. In present study we investigated the association between plasminogen and α_2-antiplasmin, and risk of myocardial infarction in 135 women with a first myocardial infarction and 134 controls. We adjusted all cardiovascular risk factors in patients and controls. We concluded that elevated α_2-antiplasmin levels are independently associated with risk of myocardial infarction in wemen, but we found no association between levels of plasminogen and risk of myocardial infarction in women.

Key words: Fibrinolytic proteins, α2-antiplasmin, Plasminogen, myocardial infarction.

Introduction

Myocardial infarction is an important cause of morbidity, mortality, and disability in the world. Patients surviving a first myocardial infarction deserve to be considered as a special group. They have a non-fatal reinfarction rate of 4-5% and a cardiac death rate of about 6% in the first year after discharge.[1]

Several investigations have demonstrated the pathogenetic role of local thrombus formation in coronary arteries at the site of a ruptured plaque.[2,3] Plaque disruption leads to platelet adhesion activation and aggregation, and thrombin generation.[2] Abnormalities of the coagulation system were identified to have an important role in coronary thrombosis.

A multicenter European study[4] and many cohort studies[5,6] on patients with angina pectoris or previous myocardial infarction showed elevated plasma levels of tissue-type plasminogen activator (t-PA), plasminogen activator inhibitor type 1 (PAI-1), von Willebrand factor, and fibrinogen, to be predictive for future coronary events in these patients. In the European Concerted Action on Thrombosis and Disabilities (ECAT) study[7], a cohort study, including patients with angina pectoris, no association was found between levels of α_2-antiplasmin and risk of myocardial infarction or sudden death, but increased levels of plasminogen were associated with an increased risk of MI. But this finding is not accordant to the role of plasminogen in fibrinolysis. In the Atherosclerosis Risk in Communities (ARIC) study[8], a population-based cohort study, a positive association between plasminogen levels and coronary heart disease was also found.

The Leiden Study, a case–control retrospective study, published in July 2010, illustrated that increased levels of α_2-antiplasmin were associated with an increased risk of myocardial infarction in men.[9] They also mentioned that there was an association between increased levels of α_2-antiplasmin and risk of myocardial infarction in men. They noticed that the plasminogen activator inhibitor type1 (PAI-1), tissue-type plasminogen activator (t-PA), and plasminogen are no independent risk factors for myocardial infarction. They concluded that plasminogen is a marker of inflammation, and

high lipid levels and sometimes inflamation can increase the plasma levels of PAI-1 and t-PA. In addition, endothelial activation may explain the association between elevated levels of t-PA and myocardial infarction.[9]

In the present study, we investigate associations between levels of plasminogen and α_2-antiplasmin, and the risk of myocardial infarction in women.

The association between established cardiovascular risk factors and these two fibrinolytic factors was studied in Leiden.[9] According to Leiden study, α_2-antiplasmin was negatively associated with age, HDL cholesterol, and systolic blood pressure, and was positively associated with total cholesterol, triglyceride,and plasminogen levels. α_2-antiplasmin also increased with BMI.[9]

They also demonstrated that plasminogen levels increased with levels of total cholesterol, triglycerides, and CRP and was increased in smokers. Similar to α_2-antiplasmin, plasminogen increased with BMI. They also found that regular drinkers of alcoholic beverages had increased plasminogen levels.[9]

In our study, confounding variables in the association between α_2-antiplasmin or plasminogen and myocardial infarction were chosen from Leiden study.

Methods

Study population

Our study is a single-center case–control study, which included 140 female patients who surviving a first myocardial infarction at least 3 month before enroling, and 140 female controls. The patients and controls were between 40 to 70 years old. Patients and controles enroled from indviduals who admited to the hospital for coronary angiography.

All of these characteristics shuld exist in the discharge report to confirm acute myocardial infarction: typical chest pain, electrocardiographic changes that indicate myocardial infarction (ST-elevation MI or non ST-elevation MI), or a transient rise in cardiac enzymes (troponin-I or CK-MB) to more than three times the upper limit of normal.

The control group consisted of 140 women without a history of myocardial infarction or acute coronary syndrom. They had not received any anticoagulation agents in the 6 months before enrolling in the study. Control subjects were matched on 10-year age groups and 3 BMI groups to the patients. They were chosen among 200 women and were matched on smoking, having diabetus mellitus and hypertention to the patients. Patients and controls completed a questionnaire on cardiovascular risk factors, including history of diabetes mellitus, hypertentiom, dislipidemia, smoking habits, and use of medication. Also, for patients, presence of diabetes mellitus before myocardial infarction was achieved from discharge report. Patients using either insulin or glucose lowering oral agents on the day of inclusion were defined as diabetics.

Pationts or controles who have a systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg, or use of anti-hypertensive medication on the day of inclusion was considered hypertensives. BMI was calculated by dividing weight (in kilograms) by squared height (in meters). All individuals partcipated in this study filled informed consent.

Laboratory analysis

Fasting blood samples were drawn under standardized conditions by venepuncture in the antecubital vein and were obtained between October 2010 and May 2012. Blood samples were drawn in the morning before 10: 00 AM. Serum and plasma samples were sent to laboratory in multiple tubes and were immediately stored at -80°C.

Plasma C-reactive protein (CRP) levels were measured using an enzyme linked immunosorbent assay (ELISA). Triglyceride, total cholesterol and HDL concentrations were measured using enzymatic assays adapted to a Hitachi 917 analyzer.

Total hemoglobin and A_1C hemoglobin concentrations were determined after hemolysis of the anticoagulated whole blood specimen. Total hemoglobin was measured colorimetrically.

A_1C hemoglobin was determined immunotubidi-metrically. The ratio of both concentrations yields the final percent A_1C hemoglobin result (Hb A_1C %) (Instrument: Cobas Integra 400, Manufacturer: Roche diagnostic, Germany).

Plasma levels of α_2-antiplasmin and plasminogen were measured in citrated plasma. Their activity was measured with the use of chromogenic assays (STA Stachrom antiplasmin and STA Stachrom plasminogen).

In 6 controls and 5 patients, the plasma levels of α_2-antiplasmin and plasminogen were not measured

because the plasma was not sufficient. Thus, 135 patients and 134 controls leaved in the analyses.

Statistical analysis

For comparing the cardiovascular risk factors (quantitative variables) between two groups, t-test was performed. These variables include age, BMI, systolic blood pressure, diastolic blood pressure, A_1C hemoglobin, triglyceride, total cholestrol and HDL cholestrol. Also, t-test was used for comparing plasma levels of α_2-antiplasmin and plasminogen between two groups. Chi-Square tests were performed to compare the cardiovascular risk factors (qualitative variables) between two groups. These variables include: having diabetes mellitus, hypertention, dislypidemia (triglyceride, total cholesterol, HDL cholesterole), BMI groups, age groups, A_1C hemoglobin and CRP. Further more, α_2-antiplasmin and plasminogen levels also compared by Chi-Square tests. SPSS 16.0 was used for statistical analyses.

Results

Mean age of the 135 patients with myocardial infarction was 58.4 years (5th-95th percentiles, 42.2-74.7 years) and mean age of 134 control subjects was 58.1 years (5th-95th percentiles, 42.4-73.7 years). Only one person of each groups was smoker and non of the participants drink alchiolic beverages.

Mean BMI of 135 patients was 27.4 Kg/m^2 (5th-95th percentiles, 19.8-35.1 Kg/m^2) and mean BMI of 134 control subjects was 27.5 Kg/m^2 (5th-95th percentiles, 19.9-34.6 Kg/m^2).

Mean diastolic pressure of the 135 patients was 79.3 mmHg(5th-95th percentiles, 64.3-94.3 mmHg) and mean diastolic pressure of 134 control subjects was 80.1 mmHg (5th-95th percentiles, 62.9-97.3 mmHg). Mean systolic pressure of the 135 patients was 124.1 mmHg(5th-95th percentiles, 101.9-146.3 mmHg) and mean systolic pressure of 134 control subjects was 125.5 mmHg (5th-95th percentiles, 99.1-151.9 mmHg).

Mean A_1C hemoglobin of 135 patients was 6.71%(5th-95th percentiles, 3.45-9.97%) and mean A_1C hemoglobin of 134 control subjects was 6.99% (5th-95th percentiles, 3.48-10.52%).

Mean triglyceride of 135 patients was 186.1 mg/dl (5th-95th percentiles, 36.71-335.5 mg/dl) and mean triglyceride of 134 control subjects was 193.8mg/dl (5th-95th percentiles, 57.1-330.6 mg/dl).

Mean Total cholesterol of 135 patients was 184.3 mg/dl (5th-95th percentiles, 85.6-282.9 mg/dl) and mean Total cholesterol of 134 control subjects was 194.3 mg/dl (5th-95th percentiles, 112.2-276.3 mg/dl).

Mean HDL cholesterol of 135 patients was 41.6 mg/dl (5th-95th percentiles, 19.7-63.5 mg/dl) and mean HDL cholesterol of 134 control subjects was 42.0 mg/dl (5th-95th percentiles, 25.3-58.7 mg/dl). (table 1.)

As it is shown in the table 1 there was no significant diffrence in cardiovascular risk factors between patients and control group as compared by t-test.

Mean α_2-antiplasmin level in patients was 806.88 ng/L (5th-95th percentile, 0-1885.54 ng/L)

Table 1. Mean characteristics for Patients and Control Subjects by Use of t-Test for comparison cardiovascular risk factors and fibrinolytic

Characteristic	Patients n=135	Control n=134	t	p-value
	mean±SD	mean±SD		
Age(years)	58.45±8.12	58.07±7.84	.387	0.699
BMI(Kg/m^2)	27.45±3.82	27.54±3.52	-.191	0.849
Systolic blood pressure(mm Hg)	124.07±11.09	125.52±13.21	-.974	0.331
Daistolic blood pressure(mm Hg)	79.29±7.48	80.11±8.59	-.830	0.407
A_1C Hemoglobin(%)	6.71±1.63	6.99±1.76	-1.381	0.168
Triglyceride(mg/dl)	186.08±74.68	193.84±68.37	-.888	0.375
Total cholesterol(mg/dl)	184.28±49.32	194.28±41.03	-1.806	0.072
HDL cholesterol(mg/dl)	41.61±10.94	42.00±8.36	-.324	0.746
α_2-antiplasmin(ng/L)	806.88±539.33	421.36±33.75	8.258	< 0.001
Plasminogen(%)	157.85±25.21	160.02±24.41	-.718	0.473

and 421.36 ng/L in controls (5th-95th percentile, 353.86-488.86 ng/L). Mean plasminogen level in patients was 157.85% (5th-95th percentile, 107.43-208.27%) and 160.02% in controls (5th-95th percentile, 111.2-208.84%). Surprisingly, mean plasma levels of α_2-antiplasmin in patients was approximately two times higher than controls. Although, the mean plasma levels of plasminogen had no significant deffrence as compared by t-test.

Another statistical analysis was performed to compare cardiovascular risk factors and fibrinolytic proteins between patients and controles. In this analysis we used Chi-square test for comparision. As it shown in table 2, cardiovascular risk factors and fibrinolytic proteins were arranged in many groups for comparing patients and controles.

We found no significant difference in cardiovascular risk factor between patients and controls in Chi-square analysis(table 2). These risk factors including age, BMI, smoking, hypertention, diabetes mellitus, A_1C hemoglobin, triglyceride, Total cholesterole, HDL cholesterole and CRP.

Table 2. Characteristics for Patients and Control Subjects by Use of Chi-square Test for Comparison cardiovascular risk factors and fibrinolytic proteins

Characteristics n		Patients n=135		Control n=134		p-value
		%	n	%		
Age(years)	40-49	26	19.3	25	18.7	0.992
	50-59	51	37.8	51	38.1	
	60-70	58	43.0	58	43.3	
BMI(Kg/m^2)	19-25.9	47	34.8	46	34.3	0.984
	26-30	59	43.7	58	43.3	
	>30	29	21.5	30	22.4	
Smoking	No	134	99.2	133	99.2	1.000
	Yes	1	0.7	1	0.7	
Hypertention	No	39	28.9	38	28.4	1.000
	Yes	96	71.1	96	71.6	
Diabetes mellitus	No	73	54.1	75	56.0	0.807
	Yes	62	45.9	59	44.0	
Hemoglobin A_1C(%)	<6.5	82	60.7	76	56.7	0.537
	≥6.5	53	39.3	58	43.3	
Triglyceride (mg/dl)	<150	50	37.0	46	34.3	0.849
	150-200	33	24.4	32	23.9	
	>200	52	38.5	56	41.8	
Total cholesterol (mg/dl)	<200	93	68.9	86	64.2	0.543
	200-240	23	17.0	30	22.4	
	>240	19	14.1	18	13.4	
HDL cholesterol (mg/dl)	<40	83	61.5	88	65.7	0.402
	40-50	42	31.1	41	30.6	
	>50	10	7.4	5	3.7	
CRP (mg/L)	<5	96	71.1	103	76.9	0.223
	5-10	15	11.1	17	12.7	
	>10	24	17.8	14	10.4	
α_2-antiplasmin (ng/L)	<480	17	12.6	132	98.5	< 0.001
	480-680	68	50.4	2	1.5	
	>680	50	37.0	0	0.0	
Plasminogen (%)	<130	16	11.8	17	12.7	0.920
	130-160	54	40.0	51	38.1	
	>160	65	48.9	66	49.3	

AS the pervious analysis, there was no significant defference in plasminogen levels between two groups, but α_2-antiplasmin levels were significantly higher in patients as compared by Chi-sqare test (p-value<0.001). Among 135 pationts with pervious myocardial infarction 17 indviduals(12.6%) had α_2-antiplasmin levels lower than 480 ng/L , 68 patients (50.4%) had α_2-antiplasmin levels between 480 to 680 ng/L and 50 patients(37%) had α_2-antiplasmin levels above 680 ng/L. Among 134 controls, 132 indviduals (98.5) had α_2-antiplasmin levels lower than 480 ng/L, only two person (1.5%) had α2-antiplasmin levels between 480 to 680 ng/L and no one had α_2-antiplasmin levels above 680 ng/L. (figure 1)

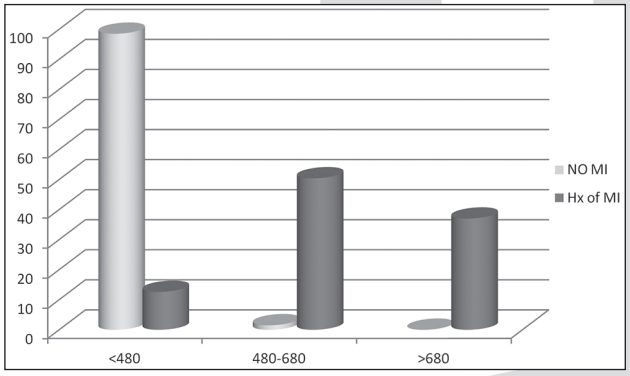

Figure 1. Plasma levels of α_2-antiplasmin in women with and without history of myocardial infarction

Horizontal axis demonstrates the plasma levels of α_2-antiplasmin (ng/L). Vertcal axis indicates percentage of patients with history of myocardial infarction and controls in each category of α_2-antiplasmin levels.

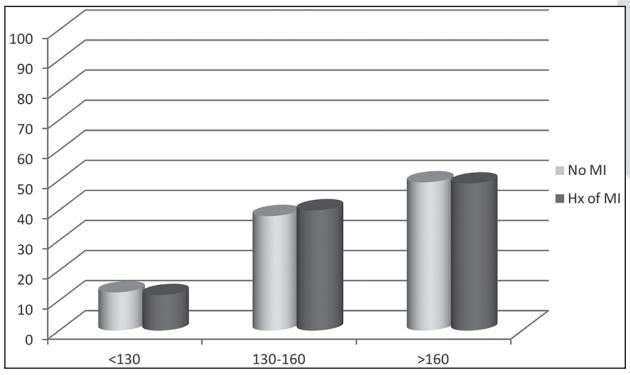

Figure 2. Plasminogen levels in women with and without history of myocardial infarction

Horizontal axis demonstrates the plasma levels of plasminogen (%). Vertcal axis indicates percentage of patients with history of myocardial infarction and controls in each category of plasminogen levels.

We found no significant difference in cardiovascular risk factor between patients and controls in Chi-square analysis (table 2). These risk factors including age, BMI, smoking, hypertention, diabetes mellitus, A_1C hemoglobin, triglyceride, Total cholesterole, HDL cholesterole and CRP.

AS the pervious analysis, there was no significant defference in plasminogen levels between two groups (figure 2), but α2-antiplasmin levels were significantly higher in patients as compared by Chi-sqare test (p-value<0.001). Among 135 pationts with pervious myocardial infarction 17 indviduals (12.6%) had α2-antiplasmin levels lower than 480 ng/L , 68 patients (50.4%) had α2-antiplasmin levels between 480 to 680 ng/L and 50 patients(37%) had α2-antiplasmin levels above 680 ng/L. Among 134 controls, 132 indviduals (98.5) had α2-antiplasmin levels lower than 480 ng/L, only two person (1.5%) had α2-antiplasmin levels between 480 to 680 ng/L and no one had α2-antiplasmin levels above 680 ng/L. (figure 1)

According to our study most patients (87.4%) with hystory of myocardial infarction had α2-antiplasmin levels above 480 ng/L, where is most indviduals (98.5%) without hystory of myocardial infarction had α2-antiplasmin levels less than 480 ng/L.

Discussion

In this study we have indicated that increased levels of α_2-antiplasmin are associated with an increased risk of a first myocardial infarction in women. This finding is accordant with established knowledge on the association between hypo-fibrinolysis and an increased risk of venus and arterial thrombosis.[10,11] The present study is the second study to show this assosiation. The Leiden study [9] is the only other study that has shown the increased risk of myocardial infarction with increasing levels of α_2-antiplasmin. The relation between plasminogen and α_2-antiplasmin levels and cardiovascular risk factors and the association between plasminogen, α_2-antiplasmin, tissue-plasminogen activator (t-PA), and plasminogen

activator inhibitor-1(PAI-1) and risk of myocardial infarction was investigated in the Study of Myocardial Infarctions Leiden which consisted of 555 men with a first myocardial infarction and 635 controls. They concluded that increased levels of α_2-antiplasmin are associated with an increased risk of myocardial infarction in men. They also showed that PAI-1, t-PA, and plasminogen are no independent risk factors for myocardial infarction. Unlike Leiden study that investigated fibrinolytic proteins in men, our study included women. Also, in Leiden study, risk factors for cardiovascular disease such as smoking, obesity, diabetes, hypertension, and hypercholesterolemia were more prevalent in patients than in control subjects. They defined quartiles of cardivascular risk factors according to the distribution among control subjects and they used Multiple linear regression to investigate which factors were independently associated with levels of α_2-antiplasmin and plasminogen. But we primarily matched patients and controls by their cardiovascular risk factors. According to table 1 and table 2 we used two statistical analysis tests to confirm that patients and controles were matched and there was no significant difference in cardiovascular risk factors between them.

In both ECAT[7] and Leiden[9] studies, they showed that plasma levels of α_2-antiplasmin were only marginally influenced by established cardiovascular risk factors. They find that although levels of most hemostatic factors increase with age, α_2-antiplasmin levels decreased with age. There was also a weak negative association between α_2-antiplasmin and HDL cholesterol and a positive association with BMI and total cholesterol. The ECAT [7] and ARIC [8] studies found a positive association between plasma levels of plasminogen and risk of arterial thrombosis. This finding contradicted to the role of plasminogen in fibrinolysis. In the Leiden study,[9] they found elevated plasminogen levels to be associated with risk of myocardial infarction, although the risk disappeared after adjustment for potential confounders. In the ECAT study patients and controls only adjusted by age, sex, and study center, and the risk of sudden cardiac death or myocardial infarction remained slightly increased after these adjustments.[7] In the ARIC study, they adjusted patients and controls for many cardiovascular risk factors, however not for CRP and triglycerides, and significant increased risks were detected even after these adjustments.[8] In the Leiden study the association disappeared after adjusting for CRP, lipid levels, alcohol use, and smoking.[9] In present study we found no association between plasminogen levels and myocardial infarction in women. We adjusted all cardiovascular risk factors in patients and controls.

In the Leiden study they found that regular drinkers of alcoholic beverages had increased plasminogen levels.[9] In our study no one of patients and controls drink alcoholic beverages. The association between lipid levels and plasminogen was described previously, but the mechanism of the association is not well known.[8] various mechanisms relating plasminogen to inflammation have been explained. Increased inflammatory state can cause increased plasma levels of plasminogen and, also, increased plasminogen levels can increase the inflammation. Because the inflammation increases the risk of myocardial infarction,[12,13] this provides evidence against a causal role for plasminogen in myocardial infarction. However, in our study there is no significant difference in plasminogen levels in women with pervious myocardial infarction and the others without an old MI.

Our study designed as a case-control study and the limitation is that plasma levels of the α_2-antiplasmin and plasminogen are measured after the event, and that levels after the myocardial infarction maybe not related to levels before the event. To aviod the acute phase reaction to interfere the plasma levels of the fibrinolytic proteins blood samples were drawn at least three month after the myocardial infarction. We adjusted patients and controls by cardiovascular risk factors including, age, BMI, smoking, hypertention, diabetes mellitus, dyslipidemia and CRP.

Our study showed that increased plasma levels of α_2-antiplasmin are associated with an increased risk of myocardial infarction in women. Also we conclude that the plasminogen is no independent risk factor for myocardial infarction in women.

However, because our study was a retrospective study, it is reasonable to desigin stronger prospective studies to investigate the association between plasma levels of fibrinolytic protoins and myocardial infarction.

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Atherosclerotic background of chronic kidney disease in sickle cell patients

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Abstract

Background: We tried to understand presence of any atherosclerotic background of chronic kidney disease (CKD) in patients with sickle cell disease (SCD).

Methods: The study was performed in the Hematology Service of the Mustafa Kemal University on SCD patients between March 2007 and June 2012.

Results: The study included 256 patients with SCD (127 females). The mean age of them was 29.3 ± 9.5 (14-59) years. CKD was detected in 8.2% (21) of the patients without any gender difference (9.3% of males versus 7.0% of females, $p>0.05$). Cirrhosis was detected in 5.8% (15) of the patients without any gender difference, too (5.4% of males versus 6.2% of females, $p>0.05$). On the other hand, there were 15 (5.8%) patients with chronic obstructive pulmonary disease with a highly significant male predominance (8.5% versus 3.1%, $p<0.001$). Digital clubbing and pulmonary hypertension were also higher in males, but the differences were non-significant (6.2% versus 4.7% and 12.4% versus 11.0%, respectively). Similarly, the leg ulcers were also higher in males, and the difference was highly significant (16.2% versus 5.5%, $p<0.001$). The significant male predominance was also observed in smoking and stroke (11.6% versus 3.9%, $p<0.001$ and 6.2% versus 3.1%, $p<0.05$, respectively).

Conclusion: CKD may not solely be a renal disease instead it may just be one of the terminal consequences of the accelerated systemic atherosclerotic process, the metabolic syndrome. SCD is an accelerated systemic atherosclerotic process, too, and the higher prevalence of CKD in SCD patients may also indicate the underlying atherosclerotic background of CKD.

Key words: Atherosclerosis, chronic kidney disease, sickle cell disease, metabolic syndrome.

Introduction

Atherosclerosis may be the major underlying cause of aging of human being, and probably it is an irreversible process that accelerated by many factors. Aging, excess weight, smoking, regular alcohol consumption, and various systemic inflammatory or infectious disorders may be the accelerating causes of the systemic process. The preventable causes of the systemic atherosclerotic process are mainly collected under the heading of metabolic syndrome (1-3). The syndrome is characterized by a group of metabolic risk factors including overweight, dyslipidemia, elevated blood pressure (BP), insulin resistance, and a prothrombotic and proinflammatory state for the development of irreversible diseases such as obesity, hypertension (HT), diabetes mellitus (DM), coronary heart disease (CHD), chronic obstructive pulmonary disease (COPD), cirrhosis, peripheric artery disease (PAD), and stroke (4,5). Similary, chronic kidney disease (CKD) is also a frequent and continuously increasing cause of morbidity and mortality in the world (6), and it may not solely be a renal disease instead it may just be one of the terminal consequences of the syndrome.

Sickle cell disease (SCD) is a chronic endothelial dysfunction that is characterized by sickle-shaped red blood cells which is caused by homozygous inheritance of the hemoglobin S (Hb S). Polymerisation of the Hb S distorts red blood cell into a sickle shape and decreases its elasticity. The polymerisation process probably takes place during whole life, although its severity may increase during stressful conditions. The abnormal shape and rigidity cause chronic endothelial destruction terminating with an accelerated atherosclerotic process that may be the main underlying cause of the significantly shortened survival in SCD patients (7-9). We tried to understand pre-

sence of any atherosclerotic background of CKD in patients with SCD in the present study.

Material and methods

The study was performed in the Hematology Service of the Mustafa Kemal University between March 2007 and June 2012. All patients with SCD were enrolled into the study. SCD is diagnosed by the hemoglobin electrophoresis performed via high performance liquid chromatography. Their medical histories including smoking habit, regular alcohol consumption, leg ulcers, and stroke were learnt, and cases with a history of one pack-year were accepted as smokers. A check up procedure including serum creatinine value on three occasions, hepatic function tests, markers of hepatitis viruses A, B, and C and human immunodeficiency virus, an abdominal ultrasonography, a Doppler ultrasonography to evaluate the portal blood flow, an endoscopy to detect esophageal varices just in suspected cases, and a computed tomography of the brain was performed. Cases with acute painful crisis, infections, or inflammatory events were treated at first, and then spirometric pulmonary function tests to diagnose COPD, a Doppler echocardiography to measure the systolic BP of pulmonary artery, and renal and hepatic function tests were performed on a silent phase. The criterion for diagnosis of COPD is post-bronchodilator forced expiratory volume in 1 second/forced vital capacity of less than 70% (10). Systolic BP of the pulmonary artery at and above 40mmHg during the silent phase was accepted as pulmonary hypertension (11). CKD was diagnosed with a continously elevated serum creatinine level which is greater than 1.2 mg/dL on a silent phase. Cases with renal transplantation were also put into the CKD group. Cirrhosis is diagnosed with hepatic function tests, ultrasonographic findings, esophageal varices, and ascites without any histologic procedure in the absence of any indication. Digital clubbing is diagnosed by determining the ratio of distal phalangeal diameter to interphalangeal diameter which is required to be higher than 1.0 and with the presence of Swamroth sign (12,13). Eventually, SCD patients with pulmonary hypertension, leg ulcers, CKD, smoking, regular alcohol consumption, cirrhosis, COPD, digital clubbing, stroke, and exitus were detected and compared between the genders. Mann-Whitney U test, Independent-Samples t test, and comparison of proportions were used as the methods of statistical analyses.

Results

The study included 256 patients with SCD (129 males and 127 females). The mean age of them was 29.3 ± 9.5 (14-59) years. There was not any patient with regular alcohol consumption. CKD was detected in 8.2% (21) of the SCD cases (Table 1). Although the higher prevalence of CKD in males, the difference was nonsignificant in between (9.3% versus 7.0%, $p>0.05$). Five of the CKD cases were on hemodialysis, and one with renal transplantation on the right side. Cirrhosis was detected in 5.8% (15) of the SCD patients without any gender difference, too (5.4% of males versus 6.2% of females, $p>0.05$). Although antiHCV was positive in two of the cirrhotic cases, HCV RNA was detected as negative by polymerase chain reaction method in both. Histological diagnosis of cirrhosis was required in none of the study cases. On the other hand, there were 15 (5.8%) patients with COPD with a highly significant male predominance (8.5% versus 3.1%, $p<0.001$). Digital clubbing and pulmonary hypertension were also higher in males, but the differences were nonsignificant in between (6.2% versus 4.7% and 12.4% versus 11.0%, respectively). Similarly, the leg ulcers were also higher in males and the difference was highly significant (16.2% versus 5.5%, $p<0.001$). The significant male predominance was also observed in smoking and stroke (11.6% versus 3.9%, $p<0.001$ and 6.2% versus 3.1%, $p<0.05$, respectively). On the other hand, there were 14 (5.4%) mortal patients during the five-year follow-up period without any gender difference (4.6% of males and 6.2% of females, $p>0.05$), and the mean ages of them were 26.8 and 31.0 years, respectively ($p>0.05$) (Table 2).

Discussion

CKD is described as the gradual loss of renal function over time, and both the frequency and complications of CKD are increasing in the world. For example, 1.9 to 2.3 million people have CKD in Canada (14). The Centers for Disease Control

Table 1. Characteristic features of the sickle cell cases

Variables	Prevalence	Mean age (year)	Female cases	Male cases	p-value
Pulmonary hypertension	11.7% (30)	30.4 ± 10.9 (19-56)	11.0% (14)	12.4% (16)	ns*
Leg ulcers	10.9% (28)	35.7 ± 7.6 (17-58)	5.5% (7)	16.2% (21)	<0.001
CKD†	8.2% (21)	36.6 ± 10.3 (19-54)	7.0% (9)	9.3% (12)	ns
Smoking	7.8% (20)	33.1 ± 9.3 (21-54)	3.9% (5)	11.6% (15)	<0.001
Cirrhosis	5.8% (15)	33.6 ± 12.5 (19-56)	6.2% (8)	5.4% (7)	ns
COPD‡	5.8% (15)	35.0 ± 8.7 (23-54)	3.1% (4)	8.5% (11)	<0.001
Digital clubbing	5.4% (14)	36.1 ± 12.1 (21-56)	4.7% (6)	6.2% (8)	ns
Stroke	4.6% (12)	32.5 ± 9.2 (17-47)	3.1% (4)	6.2% (8)	<0.05

**Nonsignificant (p>0.05) †Chronic kidney disease ‡Chronic obstructive pulmonary disease*

Table 2. Features of the mortal patients

Variables	Female cases	Male cases	p-value
Prevalence	6.2% (8)	4.6% (6)	ns*
Mean age (year)	31.0 ± 10.6 (19-45)	26.8 ± 7.1 (19-39)	ns

**Nonsignificant (p>0.05)*

and Prevention in the US found that CKD affected an estimated 16.8% of adults aged 20 years and older, during 1999 to 2004 (15). Although the developments of health services worldwide, the increased frequency and complications of CKD may only be explained by aging of the human being and increased frequency of excess weight in the world, since CKD may be one of the terminal end-points of the low-grade systemic inflammatory process, and an eventual advanced atherosclerosis is the main underlying cause of the CKD (16). The origin of the inflammation is unclear but aging, excess weight, smoking, regular alcohol consumption, and local or systemic inflammatory or infectious processes may be the major ones of the several possible causes. The inflammatory process is enhanced by release of various chemical factors by lymphocytes to repair the damaged renal tissues, especially endothelial cells of the renal arteriols. Due to the continuous irritation process of the endothelial cells in the above pathologies, prominent changes develop in the architecture of the renal tissue, since the chronic inflammatory process of the endothelial cells terminates with fibrosis, advanced atherosclerosis, and tissue hypoxia and infacts. Excess weight associated metabolic abnormalities such as hyperglycemia, dyslipidemia, elevated BP, and insulin resistance cause various cellular stresses that initiate tissue inflammation and immune cell activation, which in turn exacerbate the atherosclerotic process (17). For example, age (p= 0.04), high-sensitivity C-reactive protein (hs-CRP) (p= 0.01), mean arterial BP (p= 0.003), and DM (p= 0.02) had significant correlations with the mean carotid artery intima-media thickness (CIMT) as an indicator of systemic atherosclerosis (16). Increased renal tubular sodium reabsorption, impaired pressure natriuresis, and volume expansion because of activation of sympathetic nervous system and renin-angiotensin system and physical compression of kidneys by visceral fat tissue may be some mechanisms of the increased BP in excess weight (18). Excess weight also causes renal vasodilation and glomerular hyperfiltration that initially serve as compensatory mechanisms to maintain sodium balance due to the increased tubular reabsorption (18). However, in the long term these changes, along with the increased BP, cause a hemodynamic burden on the kidneys that causes chronic endothelial damage (19). With prolonged weight excess, there is increasing urinary protein excretion, and eventual gradual loss of nephron function that worsens with time and exacerbates HT. With the development of dyslipidemia and type 2 DM in overweight and obese individuals, CKD progresses much more rapidly (18). On the other hand, the systemic inflammatory effects of smoking on endothelial cells is already known with Buerger's disease and COPD (4,20). Increased oxidative stresses, inactivation of antiproteases, and release of proinflammatory mediators may terminate with a systemic inflammatory and eventual atherosclerotic process in smokers. The inflammatory and

eventual atherosclerotic effects of alcohol is much more prominent in hepatic endothelium probably due to the higher concentrations of its metabolites in the liver. Although some authors reported that alcohol consumption was not related with CKD (20), there may be some systemic consequences of the hepatic inflammation, particularly on the renal endothelium in regular alcohol users. Similarly, aging alone may be another cause of systemic atherosclerotic process that prevents adequate tissue repair. The prevented adequate tissue repair may be a significant cause of the increased risk of cancers in elders, since immune cells can not eradicate the malignant ones effectively. Chronic inflammatory or infectious disorders may also terminate with the accelerated systemic atherosclerotic process (21). For example, chronic HCV infection raised CIMT, and normalisation of aminotransferase levels with HCV clearance may be secondary to the reversal of lipids observed with the chronic infectious and atherosclerotic process (21). So, CKD may be considered as the renal consequence of the systemic atherosclerotic process, the metabolic syndrome. Therefore, although CKD is mainly be an advanced atherosclerotic process of the renal vasculature, there are close relationships between CKD and other consequences of the metabolic syndrome including CHD, COPD, PAD, cirrhosis, and stroke (22). For example, the most common cause of death in CKD is cardiovascular disease (CVD) rather than renal failure (23). Similarly, beside the pulmonary hypertension, leg ulcers, cirrhosis, COPD, digital clubbing, and stroke like atherosclerotic end-points, CKD is just one of the final consequences of the SCD as an accelerated systemic atherosclerotic process in the present study.

After the onset of CKD, probably CKD itself is an inflammatory and eventual atherogenic condition, too. There are several reports about the atherogenic feature of CKD in the literature (24,25), and all cause mortality increases as kidney function decreases (26). Oxidative stress induced endothelial dysfunction may have the central role in the pathogenesis of atherosclerosis in CKD (24). For example, higher factor VIII levels had the strongest association with renal function decrease (p<0.001), followed by interleukin-6 (p= 0.01) in a previous study (27). Statins may decrease proteinuria, and maintain renal function, probably by decreasing the oxidative stress on renal endothelial cells. As the renal function decreases, BP is increased due to fluid overload and vasoactive hormones produced by the renin-angiotensin system, increasing one's risk of developing HT, and eventual chronic endothelial damage. For example, vascular calcifications were seen in 56% of CKD cases, and 46% of the CKD cases had ultrasonographic criteria for atherosclerosis, which were higher in patients with HT and DM, those also showed increased susceptibility to vascular calcifications (p= 0.01) (28). Urea levels also showed significant correlation with the vascular calcifications (28). Additionally, serum CRP levels were significantly higher in hemodialysis cases (29). Cerebrovascular disease may also indicate the accelerated systemic atherosclerotic process of CKD (30), and an association between the reduced renal function and increased risk of stroke appear to be the highest in patients with dialysis (30). Similarly, CKD cases exhibited higher CIMT (p<0.0001), hs-CRP (p<0.0001), and CD4+CD28null cell frequency (p<0.0001) in another study (31). The authors also noted correlation of CIMT with age (p= 0.004) and CD4+CD28null cells as an abnormal T helper population causing endothelial injury for the development of atherosclerosis (31). There was a correlation between the atherosclerotic renovascular disease and severity of CVD in another study (32). An abnormal cardiac structure and/or function will be present in 95% of atherosclerotic renovascular disease patients, in which left ventricular hypertrophy and diastolic dysfunction are the predominant abnormalities (32), and atherosclerotic renovascular disease can be found in up to half of elders with chronic heart failure (32). Similarly, despite the minimal atherogenic effects of aging, CVD is also recognized as the major cause of death in children with advanced CKD (33). Early markers of cardiomyopathy including left ventricular hypertrophy and dysfunction, and early markers of atherosclerosis including increased CIMT, carotid arterial wall stiffness, and coronary artery calcificaton are frequent in these children (15).

Hb S causes red blood cells to change their elastic biconcave disc shape to a hard sickle shape especially during stresses. The red blood cells can take their normal elastic shapes later, but after repeated sickling and unsickling attacks, they get a permanent sickle shape with a loss of elastic motion abi-

lity that is especially important during the passage between the endothelial cells. So they harm to the vascular endothelial cells terminating with a chronic endothelial inflammation. Because of the lifelong duration of the chronic endothelial inflammation, an accelerated atherosclerotic process develops all over the body in SCD patients. Although the chronic inflammatory process is exaggerated during infections, operations, or depressions like various stresses, it is usually present during the whole lives of the patients. The chronic process is usually shown by a permanent leukocytosis and thrombocytosis even in the silent phases of the patients. The adverse effects of neutrophils on endothelium are of particular interest with regard to CHD and stroke in SCD. For example, leukocytosis during the silent phase was an independent predictor of the severity of the disease in a previous study (34), and it was associated with the risk of stroke in another study (35). On the other hand, due to the accelerated systemic atherosclerotic process, SCD may be a useful model to show the end-results of the metabolic syndrome in the early age groups (7). The very high prevalences of pulmonary hypertension (11.7%), leg ulcers (10.9%), CKD (8.2%), cirrhosis (5.8%), COPD (5.8%), digital clubbing (5.4%), stroke (4.6%), and exitus (5.4%) even in the early age group (29.3 years) may indicate some end-points of the systemic atherosclerotic process in the present study.

As a conclusion, CKD may not solely be a renal disease instead it may just be one of the terminal consequences of the accelerated systemic atherosclerotic process, the metabolic syndrome. SCD is an accelerated systemic atherosclerotic process, too, and the higher prevalence of CKD in SCD patients may also indicate the underlying atherosclerotic background of CKD.

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Relationship between CYP2A6 genetic polymorphism as a marker for nicotine metabolism and clinical parameters of chronic periodontitis

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Abstract

Background: The rate of metabolism of nicotine and its derivatives determines a greater degree of progress and intensity of destructive processes in periodontal tissue in people addicted to smoking tobacco - which proves our study.

Aim of the study: The aim of the study was to compare the clinical parameters of periodontal status among smokers and non-smokers with periodontitis in relation to CYP2A6 genetic polymorphism.

Methods: The study included 418 individuals, otherwise healthy, divided into three groups: controls; non-smokers with periodontitis; and smokers with periodontitis. All subjects were genotyped for the presence of alleles influencing CYP2A6 activity: **2, *9, *12* and for gene deletion (*CYP2A6*4*) and gene duplication (*CYP2A6*1x2*). The alleles were allocated into functional genotypes: I – normal, II – moderate reduction; III – pronounced reduction; IV – increase, in activity.

Results: There was no statistically significant association between Approximal Plaque Index (API), modified Sulcus Bleeding Index (SBI), probing pocket depth (PPD) and clinical attachment level (CAL) values, both in smokers and non-smokers regardless of genotype. In non-smoking patients with periodontitis and *CYP2A6*1*, SBI was significantly higher compared to patients with *CYP2A6 *9, *12*.

Conclusions: Smoking affects the severity of periodontitis, while the activity of *CYP2A6* affects smoking behavior and metabolism of nicotine; therefore the activity of *CYP2A6* may indirectly influence periodontitis.

Key words: CYP2A6, genetic polymorphism, nicotine metabolism, chronic periodontitis.

Introduction

Periodontitis is a multifactorial chronic inflammatory disease that targets periodontal tissues, consisting of the gingiva, the cementum, the bone lining the alveolus and the periodontal ligament, leading to irreversible attachment loss, bone destruction, pocket formation and eventually tooth loss (1). It results from a complex interaction between the subgingival microflora and nonbacterial factors, specifically host and environmental factors. The major factors determining susceptibility to periodontal disease are genetic immunoinflammatory responses and smoking habits are an important risk factor for periodontitis (2). The biological mechanism underlying the interaction between smoking and periodontal disease is very interesting. Many components of cigarette smoke affect the circulation, damaging vascular endothelial cells and triggering inflammation. Smoking habit is a behavioral risk factor for periodontal disease (2).

The National Health and Nutrition Examination Survey (NHANES) III demonstrated that smokers have a higher prevalence of periodontal sites with increased probing depth than non-smokers (3). The relative risk for smokers to develop periodontitis has been estimated to be 5- to 20-times greater than that for non-smokers (2). The combination of smoking, together with a genetic predisposition, significantly increases the risk of periodontitis (4).

While the use of tobacco products is associated with increased incidence of periodontitis and poor response to periodontal therapy (5), the direct effects of smoking on periodontal tissues are partially understood (6, 7).

Tobacco smoke contains a complex mixture of substances. Nicotine is one of the toxic products

in cigarette smoke, and has been investigated for its possible role in the process of periodontal breakdown (6). Nicotine induces local vasoconstriction, reducing blood flow and gingival bleeding. On a systemic level, smoking leads to diminished levels of circulating IgG2, the immunoglobulin that reacts with periodontitis- associated bacteria (1, 8). Nicotine, the major constituent of tobacco, is predominantly metabolized by the liver enzyme CYP2A6 into cotinine and many other compounds (9).

Several polymorphisms in *CYP2A6*, resulting in deficient *CYP2A6* enzyme activity, have been described and the relationship between nicotine metabolism and *CYP2A6* genetic polymorphisms has been demonstrated (10). Subjects who are homozygotes for *CYP2A6*4* gene polymorphism are poor nicotine metabolizers, while homozygotes for *CYP2A6*1A* polymorphism have increased nicotine metabolism (11).

Individuals with reduced or deficient activity of *CYP2A6* are less prone to smoking, and if they smoke they smoke fewer cigarettes and are less addicted to nicotine than individuals with the wild-type enzyme (12).

The aim of the study was to compare the clinical parameters of periodontal status among smokers and non-smokers with periodontitis in relation to CYP2A6 genetic polymorphism.

Materials and Methods

The study included a total of 418 individuals aged 22 to 64 years, with or without periodontal disease and otherwise healthy. Patients originating from the Western Pomerania (Poland) were included in the study after giving informed consent. The protocol of the study was approved by the local bioethics committee, and all patients gave informed written consent. All patients were not subjected to periodontal treatment or antibiotics for at least 6 months before the study.

Three groups were distinguished - the first control group consisted of 100 patients (women, W, n = 72; men, M, n = 28) with healthy periodontium aged 22 to 64 years (mean age 40.84 y). The remaining 318 patients, with periodontal disease, were divided into two groups – non-smokers and smokers. The group of non-smokers with periodontal disease consisted of 163 persons (W, n = 98; M, n = 65) aged 26-74 (mean age 50.45 y). The group of smokers with periodontal disease consisted of 155 patients (W, n = 99; M, n = 56) aged 24-71 (mean age 50.69 y).

Smokers were defined as subjects smoking at least 10 cigarettes per day for at least 5 consecutive years.

Chronic periodontitis was diagnosed using the periodontal disease classification system of the American Academy of Periodontology (1999). Chronic periodontitis was diagnosed in subjects with periodontal pockets (PPD) greater than 5 mm and clinical attachment level (CAL) greater than 3 mm in more than 20 teeth, with moderate to severe bone loss.

In all patients clinical examination was performed, confirming the presence of periodontal disease by considering the following aspects:

1. Oral hygiene condition - Approximal Plaque Index (API) according to Lange.
2. Inflammation of the gingival - modified Sulcus Bleeding Index (SBI) by Mühlemann and Son.
3. Loss of periodontal tissue by measurement (calibrated periodontal probe marked at millimeter intervals) of the periodontal pocket depth (probing pocket depth, PPD, in mm), clinical attachment level (CAL, in mm) - these measurements help determine the severity of periodontitis, and monitor progress and effectiveness of treatment.

Probing pocket depth (PPD) is the distance from the edge of the gingiva to bottom of the periodontal pocket/gingival slot. The level of the clinical attachment (CAL) - is the distance between the cement-enamel junction enamel and the bottom of the periodontal pocket. Approximal Plaque Index, according to Lange (1977) (13), expresses the percentage of interdental space with bacterial plaque. Examination was conducted after plaque was exposed in the interdental spaces using a probe. The presence of the plaque on the contact surfaces from the oral cavity side in quadrants 1 and 3, and buccal side in quadrants 2 and 4 was examined. The assessment was based only on the presence or absence of the plaque (the principle of "all or nothing") solely on the contact surfaces. The index value was given as a percentage and was calculated using the

following formula: API = (No. of plaque (+) sites / No. of sites examined) x 100. Individual API index ranges were interpreted as follows:

- API 100-70% - insufficient oral hygiene
- API 69-40% - average oral hygiene
- API 39-25% - good oral hygiene
- API <25% - optimal oral hygiene

The modified Sulcus Bleeding Index (SBI), by Mühlemann and Son (14), represents the intensity of the inflammatory response in periodontal tissue through the presence of bleeding from the periodontal pocket or gingival slot. It enables a percentage assessment and its calculation is done using the following formula: SBI = (sum of bleeding gingival units / sum of all surveyed gingival units) x 100. Periodontal status is assessed according to specified ranges of the indicator:

- SBI 100-50% - severe, generalized periodontitis
- SBI 49-20% - moderate gingivitis requiring intensive treatment
- SBI 19-10% - mild gingivitis
- SBI <10% - clinically healthy periodontium.

Patients were classified into groups of subjects based on history, physical examination and a smoking habit questionnaire. Clinical measurements were taken in homogeneous conditions in a dental clinic.

Genotyping

Genomic DNA was extracted from 200 μL of whole blood samples using GeneMATRIX Quick Blood DNA Purification Kit (EURx, Poland). All subjects were genotyped for the presence of alleles influencing CYP2A6 activity, common in Caucasians, i.e. *CYP2A6: *2, *9* and **12*. Prevalidated allelic discrimination real-time PCR assays (TaqMan, Applied Biosystems, USA) were used for detection of marker SNPs for each allele: rs1801272: T>A (c.479T>A) for **2* (Assay ID: C__27861808_60), rs28399433: T>G (g.-48T>G) for **9* (Assay ID: C__30634332_10), and rs4803380: C>T (g.5163G>A) for **12* (Assay ID: C__32350075_20). Fluorescence data was captured using real-time PCR (7500 FAST Real-Time PCR System, Applied Biosystems, USA), during 40 cycles of PCR. Genotyping for gene deletion (CYP2A6*4) and gene duplication (CYP2A6*1x2) was performed using a gene copy number assay (TaqMan Copy Number Assay, Applied Biosystems, Assay ID: Hs07545275_cn) with RNase P single-copy gene assay as the endogenous reference. In the latter method, as with the gene expression assays, measurements were made in real time. However, gene copy number assays (FAMm-labeled) use genomic DNA as template and are run as a duplex real-time (TaqMan Real-time) PCR reaction with the RNase P gene (VIC-labeled) as the reference gene to determine copy number variation.

Alleles were then classified according to their previously determined functional impact: *1 – normal activity (wild type allele); *2, *4 – non-functional allele (lack of activity); *9, *12 – reduced activity and *1x2 I increased activity, and then allocated into functional genotypes: genotype I – normal activity - *1/*1; genotype II – moderate reduction in activity: *1/*9, *1/*12, *9/*9, *4/*9, *2/*9, *2/*12, *9/*1x2, *12/*1x2; genotype III – pronounced reduction in activity *1/*2, *1/*4, *2/*2; genotype IV – increased activity *1/*1x2, *2/*1x2.

Statistical analysis

The frequency distribution of CYP2A6 genotypes was in Hardy-Weinberg equilibrium. Clinical parameters were compared between genotype groups using a Kruskal-Wallis test followed by a Mann-Whitney test. $P<0.05$ was considered statistically significant. Statistica Pl 7.1 software were used.

Results

API

The mean value of API (%) for cigarette smoking patients with periodontitis was 86.26 ± 12.12 %; for non-smoking patients with periodontitis 71.40 ± 20.10%; and for the control group API was 38.30 ± 16.05%. As shown in Table 1 there were no statistical significant associations between API and genotype.

SBI

Assessment of the modified Sulcus Bleeding Index (SBI%) of the periodontal pocket in non-smoking patients with chronic periodontitis proved to be significantly higher compared to addicted

Table 1. Comparison of Approximal Plaque Index (%), API, between four genotypes (defined in the methods); ns = not significant

Group	Approximal Plaque Index, %, ± s.d.				
	I CYP2A6 I genotype (normal activity)	**II CYP2A6 II genotype (moderate reduction in activity)**	**III CYP2A6 III genotype (pronounced reduction in activity)**	**IV CYP2A6 IV genotype (increased activity)**	**p**
Control group	38.59 ± 16.12	36.81 ± 15.81	38.33 ± 21.50		I vs II ns I vs III ns II vs III ns
Non-smokers with periodontal disease	72.30 ± 19.80	70.46 ± 20.63	65.73 ± 22.72	57.00 ± 25.46	I vs II ns I vs III ns II vs III ns
Smokers with periodontal disease	85.23 ± 13.22	87.69 ± 10.04	88.22 ± 8.86	96.00 ± 0.00	I vs II ns I vs III ns II vs III ns

Table 2. Comparison of modified Sulcus Bleeding Index (%), SBI, between four genotypes (defined in the methods)

Group	Modified Sulcus Bleeding Index, SBI, (%) ± s.d.				
	I CYP2A6 I genotype (normal activity)	**II CYP2A6 II genotype (moderate reduction in activity)**	**III CYP2A6 III genotype (pronounced reduction in activity)**	**IV CYP2A6 IV genotype (increased activity)**	**p**
Control group	8.98 ± 8.47	2.50 ± 3.76	12.67 ± 14.19		**I vs II= 0.00** I vs III ns II vs III ns
Non-smokers with periodontal disease	65.57 ± 20.25	55.96 ± 17.97	59.64 ± 20.13	54.50 ± 36.06	**I vs II= 0.02** I vs III ns II vs III ns
Smokers with periodontal disease	21.87 ± 18.56	23.02 ± 17.65	19.22 ± 11.26	27.00 ± 7.07	I vs II ns I vs III ns II vs III ns

smokers with periodontitis and those with healthy periodontium regardless of genotype (*CYP2A6 *1* allele ("normal" activity-wild-type), *CYP2A6 *9, *12* alleles (reduce activity) and *CYP2A6 *2, *4* alleles (null activity alleles).

In smoking patients with chronic periodontitis with slower nicotine clearance (*CYP2A6 *9, *12*) SBI was 23.02 ± 17.65%, with faster nicotine clearance - 21.87 ± 18.56% and with null activity - 19.22 ± 11.26% (Table 2). However, in non-smoking patients with periodontitis SBI was: 55.96 ± 17.97% for slower nicotine clearance; 65.57 ± 20.25% for faster nicotine clearance and 59.64 ± 20.13% for null activity (Table 2). The value of the modified bleeding index of the periodontal pocket (SBI) was thus significantly higher in patients with chronic periodontitis who have never smoked tobacco, p <0.0001 compared to addicted smokers with periodontitis as well as to those of control group, taking into account the activity of the *CYP2A6* genotype.

PPD

The mean value of PPD in smoking patients with chronic periodontitis with *CYP2A6*1* was 5.28 ± 1.18 mm; with *CYP2A6*9, *12*- was 4.86

Table 3. Comparison of average probing pocket depth (mm), PPD, between four genotypes (defined in the methods)

Group	Average Probing Pocket Depth, PD, (mm) ± s.d.				
	I CYP2A6 I genotype (normal activity)	**II CYP2A6 II genotype (moderate reduction in activity)**	**III CYP2A6 III genotype (pronounced reduction in activity)**	**IV CYP2A6 IV genotype (increased activity)**	**p**
Control group	1.52 ± 0.37	1.34 ± 0.22	1.60 ± 0.13		**I vs II =0,03** I vs III ns II vs III ns
Non-smokers with periodontal disease	4.48 ± 0.89	4.46 ± 0.85	4.63 ± 0.95	3.88 ± 1.18	I vs II ns I vs III ns II vs III ns
Smokers with periodontal disease	5.28 ± 1.18	4.86 ± 1.06	5.37 ± 0.93	6.15 ± 0.91	I vs II ns I vs III ns II vs III ns

Table 4. Comparison of average Clinical Attachment Level (mm), CAL, between four genotypes (defined in the methods)

Group	Average Clinical Attachment Level, CAL (mm) ± s.d.				
	I CYP2A6 I genotype (normal activity)	**II CYP2A6 II genotype (moderate reduction in activity)**	**III CYP2A6 III genotype (pronounced reduction in activity)**	**IV CYP2A6 IV genotype (increased activity)**	**p**
Control group	0.62 ± 0.87	0.20 ± 0.40	0.72 ± 0.48		**I vs II = 0.02** I vs III ns **II vs III = 0.02**
Non-smokers with periodontal disease	6.38 ± 1.75	6.67 ± 1.68	6.61 ± 1.86	5.33 ± 2.83	I vs II ns I vs III ns II vs III ns
Smokers with periodontal disease	7.66 ± 2.49	7.07 ± 2.45	7.44 ± 2.47	8.00 ± 4.24	I vs II ns I vs III ns II vs III ns

± 1.06 mm; with *CYP2A6*2, *4*- was 5.37 ± 0.93 mm. While for those non-smoking patients with *CYP2A6*1* was 4.48 ± 0.89 mm; with *CYP2A6*9, *12* was 4.46 ± 0.85 mm and with *CYP2A6*2, *4* was 4.63 ± 0.95 mm. PPD values of gingival groove depth in patients with healthy periodontium with *CYP2A6 *1, *9*, **12, *2, *4* - were significantly lower compared to other groups - addicted smokers and non-smokers with periodontitis. In this group PPD was 1.52 ± 0.37 mm in patients with *CYP2A6* *1; 1.34 ± 0.22 mm - with *CYP2A6 *9, *12* and 1.60 ± 0.13 mm with *CYP2A6 *2, *4* (Table 3).

CAL

By analyzing clinical attachment level (CAL) our research has shown significantly more increased loss in smoking patients with periodontitis. The mean clinical value of loss in smoking patients with *CYP2A6*1* with periodontitis was 7.66 ± 2.49 mm, while in non-smokers it was 6.38 ± 1.75 mm and in healthy controls 0.62 ± 0.87 mm. In individuals with *CYP2A6 *9, *12* - CAL in the control group was 0.20 ± 0.40 mm, in non-smoking patients 6.67 ± 1.68 mm, and in smoking patients 7.07 ± 2.45 mm. In the case of *CYP2A6*

**2* and **4* the clinical attachment levels were: 0.72 ± 0.48 mm in the control group, 6.61 ± 1.86 mm in non-smoking patients, and 7.44 ± 2.47 mm - in patients addicted to smoking (Table 4).

Discussion

Our study showed that the mean percentage of the API index definitely suggested poor oral hygiene in addicted smoking and non-smoking patients with periodontitis, compared to the group of people with healthy periodontium. However, in the group of smoking patients the API was significantly higher compared to patients with periodontitis non-smokers, which is consistent with the results of Tonetti (15). Oral hygiene depends on the conscientiousness of the patient and the proper brushing of teeth, and does not depend on functional genotypes.

In addition to the deterioration in oral health, patients addicted to smoking cigarettes have marked fundamental markers of inflammation such as swollen gums, redness, and bleeding from the gums during probing of the periodontal pocket. The reason for this reaction of the gums is the impact of tobacco smoke ingredients and the vasoconstrictor effects of nicotine and its metabolites (16). Nicotine stimulates the release of adrenal and peripheral catecholamines, causing vasoconstriction in the gingival surface. The formation of microthrombosis and capillary closure due to decreased levels of prostacyclin PGI2 is responsible for vasodilatation and decreased platelet aggregation (17). The clinical effect of changes in blood flow in the gums is a tendency to chronic gum diseases with a lower percentage of sites bleeding on periodontal pocket probing (17, 18, 19).

In our study, the SBI index (%) was the greatest in non-smoking patients with periodontitis compared to the other groups: patients with periodontitis who smoke and those with a healthy periodontium. This indicates that smoking has a "suppressing" effect on the symptoms of periodontal pocket bleeding, which has also been shown by Shimazaki (20). In addicted smoking patients with periodontitis, the SBI rate was the highest in those patients who had allele *CYP2A6 *2x1*, and the lowest in patients with *CYP2A6 *2* and **4*; however these differences were statistically insignificant. Both in the health group and in non-smoking patients there were statistically significant differences in SBI values between the *CYP2A6* I genotype (normal activity) and *CYP2A6* II genotype (moderate reduction in activity) with a higher SBI value for the *CYP2A6* I genotype.

Genetic variability in *CYP2A6* has been associated with a variety of smoking behavior phenotypes (21, 22). The *CYP2A6*1* allele gives the "normal" activity of *CYP2A6* (wild-type) variant, the *CYP2A6*2* and **4* alleles are null activity alleles (23) and the *CYP2A6*9* and **12* are reduced activity alleles (24).

Smokers with reduced (*CYP2A6*9,*12*) or null activity (*CYP2A6*2,*4*) smoke fewer cigarettes (25) and they are less dependent on nicotine (26, 27) than smokers with normal activity alleles (*CYP2A6*1*). Null or reduced activity *CYP2A6* alleles (*CYP2A6*2,*4,*9* and **12*) are associated with lower nicotine metabolite ratios of the nicotine metabolites- 3-HC/cotinine that is derived from cigarette smoking (28)**,** and slower metabolism (26)**.** These *CYP2A6* alleles (*CYP1A6*2, *4, *9* and **12*) are more prevalent in non-smokers than smokers (29).

In view of the fact that people with the alleles **9*, **12* have a slower metabolism of nicotine, and thus have less need for lighting a cigarette, the symptom of bleeding in these smoking patients was higher compared to patients with allele **1*, whose nicotine metabolism is faster, but who also have a greater demand for lighting a cigarette. However in our study, these differences were not statistically significant. In this regard it would be necessary to test a larger number of patients who smoke and have periodontal disease to increase the likelihood of obtaining a significant result.

Our study revealed that in patients with *CYP2A6 *1* – (faster nicotine clearance) with nicotine smokers with periodontitis, the mean depth of periodontal pockets was significantly higher than in non-smoking patients with periodontitis.

In the case of *CYP2A6 *9, *12* ('slower' nicotine clearance) and the *CYP2A6 *2, *4* (null activity), the mean value of the depth of periodontal pockets was significantly higher in smoking patients with periodontitis compared to controls.

When compared to non-smokers with chronic periodontitis, the periodontal pocket depths in smoking patients with periodontitis were deeper.

Deeper periodontal pockets in smoking patients was also shown by Shimazaki (20) and Razali (30). Only Tonetti et al. (15) observed a reduced pocket depth in smokers compared with those non-smokers with periodontitis.

Comparing PPD values in particular groups - smoking and non-smoking patients with periodontitis with respect to genotype, there were differences in PPD, but statistically insignificant. There were statistically significant differences in PPD values only in the control group between the *CYP2A6* I and *CYP2A6* II genotypes.

By analyzing the level of clinical attachment - CAL - our study showed a significantly greater loss of attachment in smoking patients with periodontitis. In the case of genotype I (normal activity) statistically significant differences have been showed between mean values of CAL in smoking patients with periodontitis and in non-smoking patients with periodontitis. Significant differences in the loss of CAL in smokers compared with non-smoking patients was also reported by Haffaje and others (31).

Some explain the grea ter loss of clinical attachment by the fact that nicotine in smokers has the ability to connec t to the surface of the tooth root (32), to cause the expression of integrins (33) and decrease collagen production but increase the concentration of collagenase (34).

In both groups – smokers and non-smokers with periodontitis regardless of genotype, there were no statistical significances between CAL values.

An interesting obser vation in our study is that patients in the control group with *CYP2A6 *9, *12* have the lowest leve l of clinical attachment loss compared to patients in the group with the *CYP2A6 *1* (normal) and *CYP2A6 *2, *4* (null activity). The differences were statistically significant.

Conclusion

CYP2A6 is responsible for the metabolism of nicotine, which is thought to affect disease activity in periodontitis. Smoking affects the severity of periodontitis, while the activity of *CYP2A6* affects smoking behavior and the metabolism of nicotine, so activity of *CYP2A6* may indirectly influence periodontitis.

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Evaluation of left atrial phasic functions in end-stage renal disease patients

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Abstract

Background: In this study we aimed to compare the left atrial (LA) volume and function of End-Stage Renal Disease (ESRD) patients on renal replacement therapy via haemodialysis (HD) or peritoneal dialysis (PD).

Methods: Thirty eight HD and 34 PD patients enrolled in the study. Left ventricle diameter, mass and the left ventricle wall thickness of all the patients was measured with M-mode echocardiography. Without visualisation of apical four chambers with two-diameter echocardiography, minimal, maximal and presystolic LA volumes measured. Via the help of these volumetric measurements LA reservoir, conduit and pump functions were calculated.

Results: The calculated LA volume in HD and PD patients were statistically significantly higher than the control group ($p<0.001$). LA functions (reservoir, conduit and pump function) of HD and PD patients were detected statistically significantly lower than the control group ($p<0.001$). It was detected that there was a significant correlation between LA functions with LV diameter and wall thickness. The LA linear diameter significantly correlated only with LA conduit function ($r=0.33$, $p=0.001$).

Conclusion: It was detected that the LA volume increased significantly in ESRD patients, while LA functions decreased significantly compared to the control group. These results were further pronounced in HD patients.

Key words: Left atrial functions, left atrial volumes, haemodialysis, peritoneal dialysis.

Introduction

Cardiovascular (CV) disease related mortality is increased in End-Stage Renal Disease (ESRD) patients [1]. Echocardiographic evaluation plays a key role in detection and stratification of cardiovascular risk in ESRD patients. Left Ventricle (LV) systolic functions, left ventricle mass (LVm), LV dilatation and LV volume are the main echocardiographic parameters that are used in evaluation and follow-up of CV risk of ESRD patients [2–5]. Left atrium (LV) volume and function are other parameters that are used to predict CV results. LA is not a sole passive chamber, it has a dynamic nature. The dynamic function of LA is evaluated in three phases: reservoir function; refers collection of pulmonary venous drainage until left ventricle contraction and iso-volumetric relaxation, conduit function; refers conduction of the collected blood to the LV in the early phase of diastole, pump function; refers active contraction at late diastole [6]. LA dilatation is related to atrial fibrillation, stroke, congestive heart failure and CV death in the general population [7]. Increased LA volume is a more pronounced predictor than the LVm and LV ejection fraction to predict a CV event in ESRD patients [8]. LA diameter and volume have to increase in ESRD patients due to long standing pressure and volume overload. Haemodialysis (HD) patients have more experience of inter-dialysis volume overload and in-dialysis volume and pressure variation compared to peritoneal dialysis (PD) patients. Also, HD patients were further exposed to haemodynamic stress. In this study we aimed to evaluate the LA volume and dynamic functions in HD and PD patients and compare the results with the healthy control group.

Methods

Patient population

Thirty eight HD and 34 PD patients who were waiting at least 6 month for renal replacement therapy were included in the study. HD patients selected from patients who were taken into haemodialysis (Fresenius 4008–S, Germany) for 4–5 hours triple in a week in our dialysis centre. The PD patients

selected were given exchanged peritoneal dialysis solution four times a day that is 1.36%, 2.36% or 3.86% in a concentration dextrose solution. The control group was comprised of 32 healthy age and sex matched individuals. The demographic properties (age, sex, present comorbid diseases, medication, etc.) of the patients were recorded. Patients with active infection, hypoalbuminemia, non-sinus cardiac rhythm, pericardial effusion, mid-severe valve disease and known coronary artery disease were excluded from the study. Local ethical committee approval and the informed consent of all patients and those in the control group were recorded.

Echocardiography

All echocardiographic measurements of the patient and control group were recorded in the left lateral position via a 2.5MHz transducer of Vingmed ultrasound system (Vingmed System 6S, General Electric, Horten, Norway). The collected record was evaluated (EchoPAC PC; GE Vingmed Ultrasound AS). Echocardiographic measurements were carried out during the pre-dialysis period of HD patients. Left Ventricle End-Systolic diameter (LVES), Left Ventricle End-Diastolic diameter (LVED), left atrium linear diameter (LA), interventricular septum diastolic thickness (IVSd) and posterior wall thickness (PW) were measured via M-mode echocardiography in the parasternal long axis [9]. LVm is calculated via the formula determined by Lang and colleagues [10]. Left ventricle ejection fraction is measured using the Simpson method.

Left atrial volumes

LA volumes were measured by the biplane area length method without apical 4 chamber visualisation [11]. Maximal volume (LAVmax) recorded at mitral valve opening time, left atrial presystolic volume (at electrocardiographic P wave=LAVpre.a) recorded at the initiation of atrial systole and minimal volume (LAVmin) recorded at mitral valve closure (Figure 1). LA functions were evaluated by following the formula via these records:

- Reservoir function: (LAVmax - LAVmin)/ LAVmax) x 100
- Conduit function: (LAVmax - LAVpre.a)/ LAVmax) x 100
- Pump function: (LAVpre.a - LAVmin)/ LAVpre.a) x 100

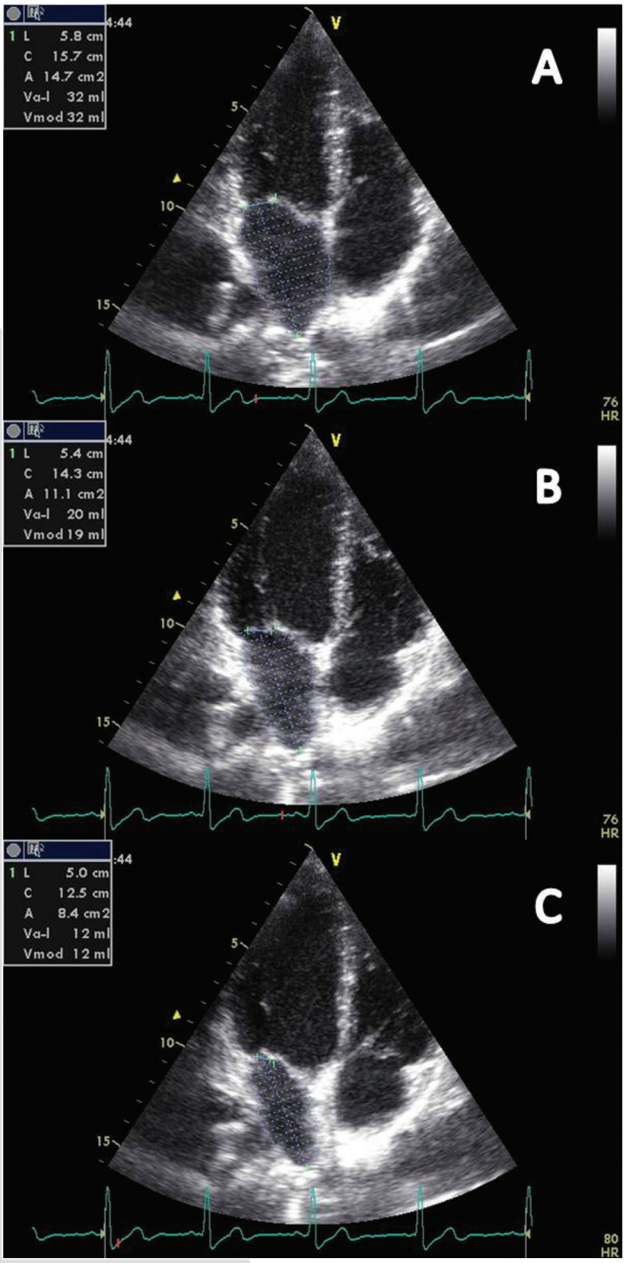

Figure 1. Examples of left atrial volumes. (A) Left atrial maximal volume at mitral valve opening time. (B) Left atrial presystolic volume at the initiation of atrial systole. (C) Left atrial minimal volume at mitral valve closure.

Statistical analysis

SPSS 20.0 statistical analysis software (SPSS Inc, Chicago, IL, USA) was used to evaluate variables and tests. Continuous variables were expressed as mean (±) standard deviation (SD). A one-way ANOVA test was used to compare the normally distributed continuous variable between the patients with dialysis and the control group. A Tukey test was used for post-hoc analysis after performing ANOVA test. Pearson correlation

analysis was used to evaluate the relation of LA functions with LA linear diameter, LVED diameter, LVES diameter and LVm. A p value less than 0.05 was considered to be statistically significant.

Results

Demographic, clinic and echocardiographic properties of the control and patient groups are presented in Table 1. Patients and the control group were similar for age, sex, body mass indexes (g/m²), duration of renal replacement therapy (month), presence of co-morbid disease and medication. Systolic and diastolic blood pressures were similar in patient groups. LVED, LVES and LA linear diameters are statistically significantly higher than both the PD and control group ($p=0.007$; $p<0.001$, $p<0.001$, respectively). Besides this, the LA diameter of PD patients is calculated as being statistically significantly higher than the control group ($p<0.001$). IVSd and PW thicknesses were significantly higher in HD patients than PD patients and the control group ($p<0.001$ and $p<0.001$, respectively). IVSd and PW thickness were similar in the PD and control group. LVm was measured as being significantly higher in HD and PD patients than the control healthy subjects ($p<0.001$). There was no statistically significant difference for the ejection fraction between the groups.

It was detected that the maximum volume index (ml/m²), minimum volume index (ml/m²) and presystolic volume index (ml/m²) of HD and PD patients were statistically significantly higher than the control group. Calculated left atrial reservoir, conduit and pump function of HD and PD patients were statistically significantly lower than the control group ($p<0.001$). In addition, it was detected that left atrial functions of HD patients was statistically significantly lower than PD patients (Table 2).

In correlation analysis, the relation between the LA function with LVm, LVED, LVES and LA linear diameter were evaluated (Table 3). It was found that all three LA function was statistically significantly correlated with LVm, LVED and LVES. LA

Table 1. Demographic, echocardiographic variables of HD, PD patients and the control group.

Variables	HD (n=38)	PD (n=34)	Control (n=32)	P-value
Age (years)	47±7.1	45.6±8.6	48.3±6.5	0.15
Sex (F/M)	18/20	16/18	15/17	0.72
BMI (kg/m2)	23.8±3.3	23.4±3.5	22.7±2.9	0.54
Duration of dialysis (month)	48±21	44±22	-	0.41
Hypertension (%)	63	59	-	0.62
Diabetes mellitus (%)	26	24	-	0.54
ACEI/ARB (%)	45	44	-	0.80
Calcium channel blockers (%)	42	44	-	0.72
Beta-blocker (%)	32	35	-	0.51
Diuretics (%)	26	24	-	0.60
SBP(mmHg)	133±16	127±17	125±15	0.18
DBP(mmHg)	80±11	78±9.1	76±8.2	0.34
LVED (mm)	50.4±4.3*$^{\delta}$	47.1±6.9	46±3.5	0.007
LVES (mm)	35.4±4.7*$^{\delta}$	32.5±6.5	30±2.6	<0.001
IVSd (mm)	13.2±1.7*$^{\delta}$	10.6±1.5	9.7±1.3	<0.001
PW(mm)	13±1.3*$^{\delta}$	11.3±2.3	10.9±1.9	<0.001
LVEF Simpson (%)	61.3±8.4	60±7.7	63.8±6.3	0.16
LA (mm)	41.1±3.5*$^{\delta}$	38.5±5.5$^{\delta}$†	34.4±2.8	<0.001
LVm (g)	244±56*$^{\delta}$	197±67$^{\delta}$†	147±22	<0.001

**-p<0.05 compared with PD, †-p<0.05 compared with HD, $^{\delta}$p<0.05 compared with control*
HD=Haemodialysis, PD=Peritoneal Dialysis, BMI=Body Mass Index; DBP: Diastolic arterial tension; IVSd=interventricular septum end diastolic; LA=Left atrium; LVED=Left Ventricle End-Diastolic diameter; LVEF=Left Ventricle Ejection Fraction; LVm=Left Ventricle Mass; LVES=Left Ventricle End-Systolic diameter; PW=Posterior Wall; SBP=Systolic arterial tension.

Table 2. Left atrium volume and functions of HD, PD patients and the control group

Left atrium	Haemodialysis (n=38)	Peritoneal dialysis (n=34)	Control (n=32)	P-value
Maximum volume index (ml/m²)	38.8±5.9*δ	35.2±4.4†δ	25.1±2.3	<0.001
Minimum volume index (ml/m²)	19.1±1.9*δ	17.1±2.9†δ	12.3±1.7	<0.001
Presystolic volume index (ml/m²)	30.5±3.2*δ	27.8±3.3†δ	18.8±2.2	<0.001
Reservoir function (%)	44.4±8.5*δ	49.7±9.4†δ	56.8±4.1	<0.001
Conduit function (%)	18.7±4.1*δ	21.2±5.8†δ	30.4±3.7	<0.001
Pump function (%)	34.9±7.2*δ	39.3±8.7†δ	44.5±4.4	<0.001

**-p<0.05 compared to PD, †-p<0.05 compared to HD, δp<0.05 compared to the control*

Table 3. The correlation analysis of left atrium phasic functions with Left Ventricular Mass (LVm), Left Ventricular End-Diastolic diameter (LVED), Left Ventricle End-Systolic diameter (LVED) and Left Atrium (LA) linear diameter

Variables	LVm (gr)	LVED (mm)	LVES (mm)	LA (mm)
Reservoir function	0.24*	0.21*	0.21*	0.11
Conduit function	0.41*	0.28*	0.32*	0.33*
Pump function	0.25*	0.22*	0.24*	0.16

**P<0.05*

linear diameter significantly correlated only with LA conduit function (r=0.33; p=0.001). The correlation between LA linear diameter with LA reservoir and pump function was statistically insignificant (r=0.11, p=0.29, r=0.16, p=0.11, respectively).

Discussion

In this study we detected that; LA volume indexes of HD patients higher than PD patients, LV diameters, LVm and LV wall thicknesses of HD patients higher than PD patients. We also detected that the LA phasic functions of PD patients is higher than HD patients.

Volume overload, hypertension and accompanying left ventricle hypertrophy are present at a very high rate in renal failure patients. Left ventricle hypertrophy has a high prognostic importance. These ratios are especially high in HD patients [12, 13]. Inter-dialysis volume overload and incidental blood pressure and volume alterations are common in classic HD patients (3 to 4 times HD in a week), incidentally LV hypertrophy is more commonly observed in HD patients. Chan and colleagues demonstrated that nocturnal HD (8-10 hours all nights) significantly decrease LVm and LV hypertrophy compared to classic HD (3 to 4 times a week) [14]. Similarly, Fagugli and colleagues demonstrated that daily haemodialysis (6 times a week, 2 hours a day) significantly decreases LVm [15]. In our study HD patients were treated with classic HD (3 times a week) and their LVm and LV wall thicknesses were significantly higher than PD patients. Our study results promote the literature.

LA phasic functions are calculated via LA volumes and affected by many pathological conditions. LA enlargement is emerging as an early adaptive response to suspend cardiac flow rate. If LA enlargements lasts long enough, Frank-starling relation in LA starts to deteriorate and leads to impairment of LA functions [16, 17]. Factors that affect LV functions will affect LA functions at the same time. The conditions that increase LA overload lead to increased LA pressure and enlargement [18]. There is a close relation between LV hypertrophy and LA dilatation and dysfunction [8, 19]. In our study LA diameter and volume increased both in HD and PD patients. This augmentation is further promoted in HD patients that had LV hypertrophy. Similarly, LA functions calculated on the basis of LA volume; is further decreased in HD patients. Although HD and PD group patients had a similar systolic and diastolic blood pressure rate, hypertension incidence, antihypertensive medication, the LA volume of HD patients was higher than PD patients and HD patients had worse LA functions than PD patients. We speculated that this difference is due to ex-

posed more volume overload of HD patients. Due to this reason, more frequent dialysis sessions may be planned for HD patients and detected complications in order to enable physicians to develop different treatment strategies.

When we investigated the relationship between LA functions and LVm, LVED, LVES and LA linear diameter, we detected that LA phasic functions strongly correlated with LVm, LVED and LVES. In evaluation of the relation of the LA phasic functions and LA linear diameter, the LA linear diameter statistically significantly correlated only with the LA conduit function. We could not to detect a significant correlation between the LA linear diameter and LA reservoir and pump function. However, LA volume measurements may allow a more accurate evaluation than LA linear measurements [11]. In our study, we detected a close relation between LV echocardiographic parameters and LA phasic functions, as we had predicted.

Limitations of the study

One of the important limitations of our study is the small number of patient groups that were included in the study. The second limitation is predialysis evaluation of HD patients when they had volume overload. However, the LA volume and functions can be evaluated independently from haemodynamic overload [20]. Another limitation of this study is lack of evaluation of volume overload of patient groups. However, according to our knowledge there is no commonly approved standard method for this evaluation.

Conclusion

Left ventricle diameter wall thicknesses increased and LA phasic functions decreased in ESRD patients. These findings are further prominent and more common in HD patients than PD patients. Taking this situation into account may contribute to degrade development of CV complications and manage treatment protocols before further deterioration of LA functions.

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Dialysis modality as a risk factor in P wave dispersion

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Abstract

Aim: Increased P wave dispersion (PWD) has been found to be associated with paroxysmal atrial fibrillation. Our aim was to assess the factors affecting P wave duration/dispersion to compare dialysis end stage renal disease patients (ESRD) on hemodialysis (HD) and continuous ambulatory peritoneal dialysis (CAPD).

Methods: Eighty five patients on HD and 51 patients on CAPD were enrolled in this study. The difference between maximum and minimum P wave duration was calculated and defined as PWD ($P_d = P_{max} - P_{min}$). Possible PWD risk factors such as age, dialysis duration, hypertension, modality of dialysis, serum electrolyte, albumin, c-reactive protein and hemoglobin levels were considered. The analysis was evaluated with two parameters; the interdialytic weight gain and mean blood pressure readings of the patients.

Results: Minimum P wave duration and PWD were negatively correlated in all patients. PWD was significantly higher in hemodialysis than the CAPD patients. PWD was positively correlated with systolic and diastolic blood pressures. The correlation of PWD with blood urea nitrogen, uric acid, potassium was accurate. There isn't any corelation between the PWD and ACE / ARB use in HD patients. When P wave dispersion risk factors were assessed with multivariate linear regression, statistically significant differences was found in dialysis modalities (β=-7.499, $p<0.0001$).

Conclusions: Apart from the well-known risk factors, HD therapy modality can cause changes in P wave duration/ dispersion and increase the risk of AF. Probably due to more physiologic continuous ultrafiltration, electrolyte balance and blood pressure control CAPD therapy should be the preferred dialysis modality in high risk patients for AF.

Key words: P wave dispersion, p wave duration, hemodialysis, continuous ambulatory peritoneal dialysis.

Introduction

Epidemiological reports have suggested that hypertension, male gender, heart failure, age, valvular heart disease, diabetes mellitus, ischemic heart disease, and disorders of the thyroid, lung, and pleura are the independent risk factors for the development of atrial fibrilllation (AF). The prevalence of AF in patients with chronic renal insufficiency is three times more common than in the general population. This decrease may be associated with hypertensive, ischemic and valvular heart diseases, pericarditis and dilated cardiomyopathy, diabetes mellitus, ionic disturbances, and autonomic dysfunction in ESRD patients [1].

P wave dispersion constitutes an important contribution to the field of non-invasive cardiology and is defined as the difference between the longest and shortest P wave duration recorded from surface electrocardiogram (ECG) lead [2]. The P wave occurring by the spread of excitation from the sinoatrial node over the atrial musculature represents the depolarization of the atrium. On account of the reentran nature of AF, there could be areas in the atria with different conduction properties for biggining of AF, and prolongation in PWD could reflect both intra- and interatrial heterogeneity in the conduction of sinus nodal impulses. There for, prolonged PWD can be used as a predictor of the development of AF in various clinical settings [3].

The response of P wave duration and PWD İn ESRD patients may be variable and mediated by an interplay of electrolytic shifts and electrophysiologic and extracardiac mechanisms associated with the alleviation of fluid overload [4]. There are few data

about P wave duration and P dispersion in dialysis patients. Majority of these studies were performed on HD patients, and the effect of PD on these P wave characteristics has not been widely examined. One study (in hemodialysis patients) PWD remained stable [5], while others reported either increase [6, 7] or even decrease in PWD [8]. There is a few number of studies investigating the effect of HD and CAPD on PWD [9]. The aim of our study was to compare the dialysis modality on PWD and to analyze the determinants of PWD in HD and CAPD patients.

Materials and methods

The study was approved by the local ethics committee and all participants gave informed consent. Patients on HD and on CAPD were enrolled and followed up in Baskent University Faculty of Medicine Hospital. 2000-3000 ml dialysate solutions (Baxter) were infused to CAPD patients 4-5 times a day at least for the last one year. All HD patients were dialyzed on a 4-5 hour at least for the last one year, 3 times weekly and mean Kt/V maintained at >1.4 during each treatment. We performed a mini course for education to each patient and their family about initial modality selection. Patients and their families decisions played a pivotal role in choosing the modality. Patients desire and motivation is one of the most important indication for selection of CAPD or HD. Our center let them to choose the modality unless it was not contraendicated.

Exclusion criteria were significant impulse generation or conduction defect, autonomic or metabolic abnormality, any medical history of AF, valvular heart disease, ischemic heart disease, disorders of the thyroid, lung and pleura, rheumatic cardiac valvular disease, central venous hemodialysis catheters, those who had been taking antiarrythmic drugs, no P wave on ECG, or no clear point of return to the isoelectric line. No changes were made regarding antihypertensive agent choices during the studies.

All subjects underwent 12-lead ECG was recorded at a paper speed of 50 mm/s and 2 mV/cm immediately before dialysis. On every occasion, the ECG was obtained in a comfortable supine position. During ECG recordings, all patients breathed freely and did not speak. For measurement of P wave duration, the 12-lead ECG printouts were enlarged on the same photo-copier. P wave duration was measured with calipers by one observer in order to exclude inter-observer variability. P wave duration was measured from the first electrical activity to the offset at the junction between the end of P wave deflection and the isoelectric line. The difference between maximum and minimum P wave duration was calculated and defined as PWD ($P_d = P_{max} - P_{min}$).

Statistical analyses were done with SPSS for Windows (SPSSInc., Chicago, IL, USA) statistics programme. The parametrical variables were expressed as mean ± SD (standard deviation) and non-parametrical variables were expressed as median, (min.-max). Chi-square test, Student's T test, Mann-Whitney U Test, Pearson Correlations and Spearman Correlations were used, $p< 0.05$ was considered significant. The factor effecting PWD are evaluated with linear regression analysis.

Results

Eighty five (mean age: 49.64 ± 11.68 years; male 44; mean dialysis duration: 121.56 ± 69.34 months) patients on HD and 51 (mean age: 45.71 ± 11.92 years; male 28; mean dialysis duration: 100.11 ± 56.53 months) patients on CAPD were evaluated in this study. The characteristics of patients were illustrated in table 1. PWD was significantly higher in HD than the CAPD patients (35.03 ± 4.06 ms, 27.88 ± 2.2 ms respectively; $p<0.001$). PWD was positively correlated with serum uric acid ($r=0.227$, $p=0.008$), potassium ($r=0.269$, $p=0.002$), BUN ($r=0.281$, $p=0.01$). No correlation was observed between serum calcium and PWD. There was not corelation between the PWD and ACE / ARB use in HD patients.

Serum hemoglobin level is higher in CAPD patients than HD. Serum sodium, potassium, albumin, iPTH, uric acid and ferritin levels are higher in HD patients than CAPD. (table 1). One hemodialysis patients used allopurinol and CAPD patients don't use allopurinol. Serum calcium levels were higher in patients with p dispersion positive than negative ($p<0.05$). 21 one of hemodialysis patients were administered Angiotensin Converting Enzyme Inhibitor (ACEI) (19%), in 16 (14%) patients angiotensin receptor blokers (ARB). The antihypertensive agent usage of CAPD patients were as follows: 13 patient ACEI (16.25%) and 30 (37.5%) patients angiotensin receptor blokers. CAPD patients were us-

Table 1. Demographic and Laboratory Characteristics of Study Patients

	HD patients (n=85)	CAPD patients (n=51)	P
Mean Age, y	50.8±12.4	45.7 ±11.9	NS
Gender, M/F	45/45	28/23	NS
Mean duration of dialysis, mo	125.5±69.8	100.1 ±56.5	NS
Systolic blood pressure, mmHg	145.45±30.02	126±28.75	NS
Diastolic blood pressure, mmHg	95.34±11.32	87.67±25.67	NS
BUN, mg/dL	74.11±14.45	64.90±15.33	NS
Creatinine, mg/dL	10.15±2.59	10.90±2.10	NS
Sodium, mmol/L	138.04±2.51	137.41±3.67	p<0.05
Potassium, mmol/L	5.22±0.79	4.44±0.63	p<0.05
Calcium, mg/dL	9.12±0.92	9.31±0.74	NS
Phosphorus, mg/dL	5.12±1.53	5.18±1.21	NS
Uric acid, mg/dL	6.47±1.27	5.58±1.66	p<0.05
Total cholesterol, ,mg/dL	153.28±41.82	212.54±42.52	NS
LDL, mg/dL	84.73±26.93	123.92±27.41	NS
Triglyceride, mg/dL	182.75±32.65	229.43±33.72	NS
Albumin, g/dL	4.15±0.26	3.59±0.67	p<0.001
CRP, mg/L	9.30±8.75	7.67±7.28	NS
iPTH, pg/mL	540.05±57.55	408.15±41.63	p<0.001
Hemoglobin, g/dL	11.06±1.66	13.36±1.99	p<0.05
Serum iron levels, ug/dL	57.96±29.91	72.61±33.15	NS
Iron binding capacity, ug/dL	171.47±84.77	203.65±80.26	NS
Ferritin, ng/mL	415.17±266.96	351.63±207.66	p<0.05
P maximum duration, ms	98.88±5.85	95.16±5.16	NS
P minimum duration, ms	63.60±6.52	66.60±5.95	NS
P wave dispersion, ms	35.05 4.1	27.88 2.2	p<0.001

Table 2. Factors Affecting P Wave Dispersion: Multivariate Regression Analysis

Variable	Relative Risk	95%CI		P Value
Dialysis Modality	-7.499	-8.893	- 6.105	0.0001
Potassium, mmol/L	-0.429	-1.215	- 0.357	0.283
Uric Acid, mg/dL	0.001	-.399	- 0.422	0.956

ing ACEI and angiotensin receptor antagonist more than hemodialysis patients (p<0.05).

When P wave dispersion risk factors were assessed with multivariate linear regression, significant differences was found in dialysis modalities (β=-7.499, p<0.0001). We demonstrated a poor correlation for the value of R<25 (Table 2).

Discussion

In this report, 85 patients on HD and 51 patients on CAPD were evaluated. P wave duration and dispersion were calculated from a 12-lead surface ECG. In our study, we demonstrated that PWD is increased in HD patients than CAPD patients.

Hemodialysis is associated with a significantly higher risk of atrial fibrillation than peritoneal dialysis in one study [10] and other study demosrtrated that PWD is more frequently in hemodialysis patients than CAPD patients [9]. It is possible this association represents bias; since hemodialysis patients present for medical care much more frequently than peritoneal dialysis patients and progressive cardiac enlargement was more frequent in hemodialysis patients than peritoneal dialysis patients [11]. Also, the typical regimen for hemodialysis is likely far less physiologic than peritoneal dialysis [12]. Some or all of these factors may therefore mediate the higher risk of PWD associated with hemodialysis.

Anemia are known to induce left ventricular hypertrophy [13]. In addition to, LVH lead to heterogeneity-instability in atrial conduction and PWD [14]. PWD was less in CAPD patients than hemodialysis patients. Maybe, high ferritin and low hemoglobin levels trigger this situation in hemodialysis patients.

The studies show that the relative risk of death in patients on HD versus PD changes over time with a lower risk on PD. The survival advantage of PD continues for 1.5-2 years but, over time, the risk of death with PD equals or becomes greater than with HD. Thus, PD survival is best at the start of dialysis [15]**.** In addition to, Hemodialysis itself through changes in electrolyte levels might create a milieu relatively arrhythmogenic in ESRD [1]. Hyperkalemia affects the myocardial tissue producing electrocardiographic abnormalities, such as prolongation of the P-R interval, tall peaked T waves, a reduction in the amplitude and an increase in the duration of P wave, and atrial and ventricular arrhythmias [16]. In one study Dr. Tezcan and colleagues showed that, there was a significant decrease in the potassium levels at the end of dialysis and negative correlation was found with serum potassium and PWD [6]. But in our study, PWD was positively correlated with serum potassium levels in all patients.

In recent years, the studies indicating increased morbidity and mortality associated with high serum calcium levels (>10.0 mg/dl) among long-term HD patients. In addition to in hemodialysis patients cumulatively low calcium levels (<9.0 mg/dl) are associated with higher death risk (17). As is known; intracellular calcium is the main stimulator of contraction of smooth muscle and cardiac muscle, and are required for the formation of the impulse in sinoatrial cells. Tezcan et al. suggest that; PWD was positively correlated with serum calcium levels after at the end of the dialysis [6]. In accordance to this study serum calcium levels was high in the patients with positive PWD.

In our study, uric acid is higher in HD patients than in CAPD. In one study, uric acid is a toxin to the heart [18]. Uric acid might determine lengthening of cardiac depolarization and in present study, the PWD of HD patients may be related to high uric acid levels of the HD patients.

In our study; PWD was negatively correlated with serum iron levels and ferritin was enlarged in hemodialysis patients than CAPD patients. Over iron levels in cardiac muscles may be affect cardiac conduction and PWD. The better hemoglobin control in peritoneal dialysis patients maybe affect to this situation.

In one study; thirty eight newly diagnosed hypertensive patients and randomly assigned to receive treatment with either irbesartan (150-300 mg) or quinapril (20-40 mg) it was demonstrated that both drugs significantly reduced blood pressure to a similar degree ($P<0.001$) and antihypertensive treatment with either irbesartan or quinapril is associated with significant reductions in Pmax and PWD [19]. ARBs and ACEIs have beneficial effects on atrial conduction times [20]. In our study; hemodialysis patients PWD was found significantly higher than CAPD patients and ARBs-ACEIs drugs use were grater in CAPD patients. Probably, difference between HD and CAPD patients for PWD related that ACEIs and ARBs drugs use over in CAPD patients than hemodialysis patients. Furthermore, systolic and diastolic blood pressure were grater in hemodialysis patients than CAPD. The worse blood pressure control and lower ACEIs and ARBs drugs use in hemodialysis patients maybe affect to this situation.

Study Limitations

In our study the first limitation, P wave duration was calculated manually, measurements were performed with calipers and magnifying lens. However, Dilavers et al.,[21] comparing three different methods for the manual measurement of P wave duration, the lowest errors were associated with the onscreen measurement. Therefore, also in our study on-screen measurements would have produced much fewer intra-interobserver relative errors. The other limitation of our study are the lack of data about magnesium and echocardiography.

Conclusion

End stage renal dissease patients harboring well recognized risk factors for AF in the general population might be under greater risk than their counterparts induced by the hemodialysis itself and/or the ionic imbalance. Our findings showed significant increase in PWD on hemodialysis patients at the beginnings of hemodialysis, which

possibly reflects the increased susceptibility of hemodialysis patients to AF. CAPD therapy may be a better dialysis modality in high risk patient group for physiologic continuous ultrafiltration, electrolyte disorders and blood pressure control. The prediction of atrial arrhythmias in ESRD patients should be evaluated in prospective studies.

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Anatomical features and relations of intervertebral discs and scoliotic curve before and after chirurgical treatment

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Abstract

The anatomical parameters caused scoliosis before and after chirurgical treatment were the objective of this research.

Corpulence of intervertebral discs close by apical vertebra, of convex and concave side of curve and Cobb angle before and after chirurgical treatment were measured.

Correlation between measured values of anatomical parameters before and after chirurgical treatment, at the end of the treatment (6-12 mounts after operation) respectively, was established.

The patients were operated by chirurgical treatment of back towards with ISOLA and SSE utensil (the same utensil and method).

Increased percentage (≈90%) in the upper and lower apical disc thickness on the concave side of the curve was noted after operation. The correlation factor of the Cobb angle value before and after operation were very high. It means that the Cobb angle, diminished in 62,57%, could expect after this chirurgical treatment.

Key words: scoliosis of vertebral column, intervertebral disc

Introduction

Scoliosis develops usually in the course of human growth and starts with spinal rotation followed by so called primary curve. Secondary curve appears compensatory and it can be situated above or below the primary one. The most rotated spinal vertebra – and the most distant from vertical axis of the body is the apical vertebra, while the cranial i.e. proximal utmost vertebra is the one whose upper terminal surface is most curved toward the concavity of the curve, and the caudal or distal one is the vertebra whose lower terminal surface is most curved toward the concavity of the curve. Anatomical changes in spinal vertebra are most expressive in the proximal, distal and apical vertebra concerning the rotation and changing the height of vertebrae bodies, more in frontal than in sagittal plain, particularly on concave side of the curve (1,2)

In physiologic conditions, the thickness of intervertebral discs increases going from the neck to the lumbar portion of the spine, so it can reach the thickness of 15 or 20 mm between the fifth vertebrae and the first segment of sacral bone (3,4). The frontal curve of lumbar portion of the spine corrects to some extent lordosis of the lumbar portion, and when the frontal curve is in function, kyphosis of the lumbar portion can be developed, what often causes damage and prolapse in vertebral disc (5). Elastic bands and vertebral arches are considered the back stability factor of the spine, while the lateral enlargements with their bands, and intervertebral joints are the lateral stability factors of the spine (6).The biggest mechanical, particularly sagittal instability, the spine shows in its thoracolumbar portion. According to the Lethe's results (1963) 51,7% of all spinal fractures occurred in the region between the 12th chest vertebrae and the 2nd lumbar vertebra. (7)

Disturbance of the statical parameters for scoliosis leads to disturbance in relations of stability factors of the spine and intervertebral discs, particularly in the apical portion of the curve. That is why *the objective* of our study was to measure the height of intervertebral discs on convex and concave side of the curve in the apical vertebrae, to measure the Cobb's angle, and to determine

the relations between the measured values before and immediately after the chirurgical treatment, as well as after the completion of a patient's treatment (6-12 months after the operation).

Respondents and Methods

The study involved 24 patients randomly chosen, out of total number of 72 patients operated in the period from 1996 to 2005 year. These patients were exposed to surgical correction of scoliosis by backside access applying instrumentation according to ISOLA and SSE (Spine System Evolution). Both instrumentations, as well as surgical technique are identical. Patients were observed starting from the preoperative stage, then postoperative phase to the completion of the treatment (6-12 months after the operation) on the Department of Ortopedy and Traumatology of UKC Tuzla. During that period patients were exposed only to surgical treatment.

We analyzed radiograms of preopertive and postopertive stage of the spine of the patients for whom we measured thickness of intervertebral discs on convex and concave side of the curve in the apical vertebrae. Cobb's method was used to measure the size of the spine curve. Anterior-posterior (A-P) radiograms of all the patients were recorded in vertical posture (standing position) from the same distance.

For statistical analysis of the collected data we used basic tests, like descriptive statistics (determination of the mean, maximal, and minimal values of the sizes measured, standard deviation), correlation matrices, i.e. the test of correlation among the measured sizes where the correlation coefficient was determined with statistical significance of $p < 0,05$ ("Stat.Soft 6")

Results and Discussion

All of patients were with dextroscoliosis. The height of the intervertebral disc above the apical vertebrae, i.e. the upper apical disc was for 48,97% smaller on concave than on convex side of the curve. Immediately after the operation the height of this disc decreased both-sided, on convex side for 4%, but on concave side for 90%, so the average thickness of this disc remain smaller on concave side for 11,76% than the one on the convex side (Figures 1. and 2.).

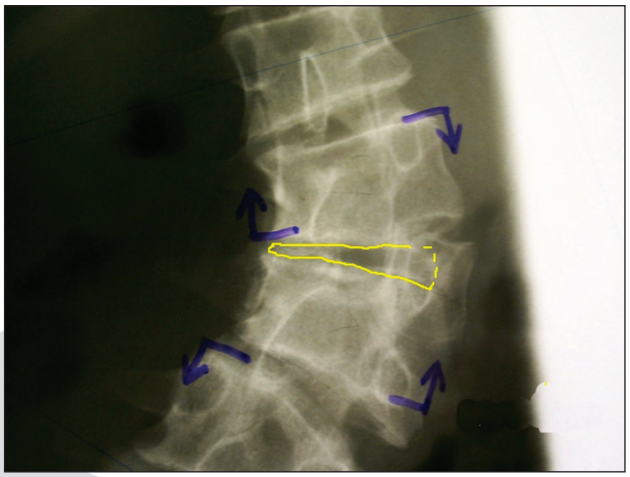

Figure 1. The thickness of intervertebral disc above the apical vertebra (example)

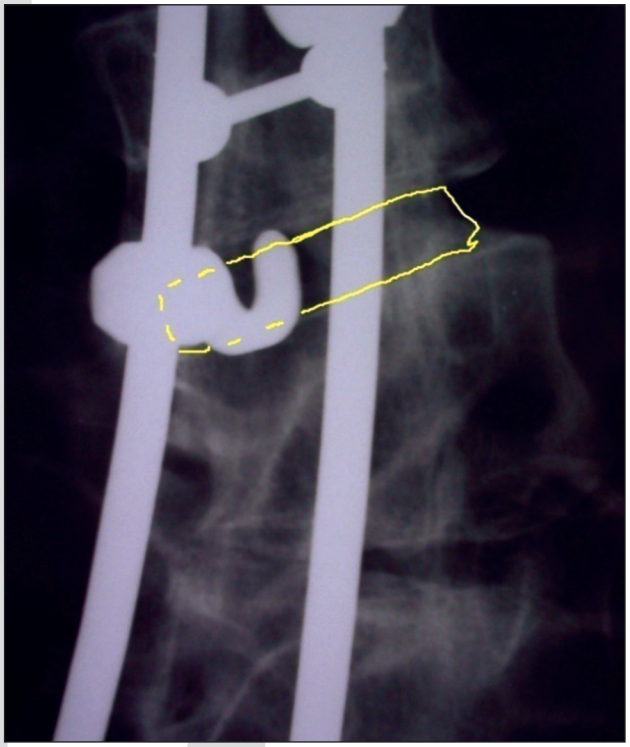

Figure 2. The thickness of intervertebral disc above the apical vertebra after the operation (example)

The height of the intervertebral disc below the apical vertebrae, i.e. the lower apical disc was for 72,41% smaller on concave than on convex side of the curve. Immediately after the operation the height of this disc decreased on the convex side of the curve for 2%, and on the concave side it increased for 91,66%, so the average thickness of this disc remained smaller on the concave side for 10,20% than the one on the convex side of the curve (Figures 3. and 4.). At the end of the treatment

insignificant decrease in the thickness of this disc was noticed for the everage of 0,02 cm on the concave side of the curve (Table 1).

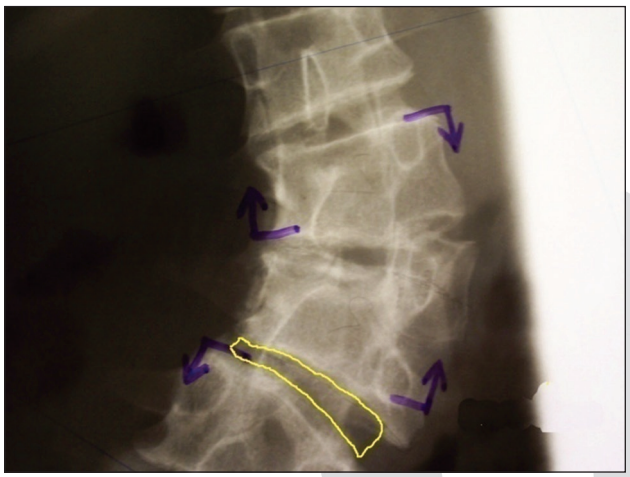

Figure 3. The thickness of intervertebral disc below the apical vertebra (example)

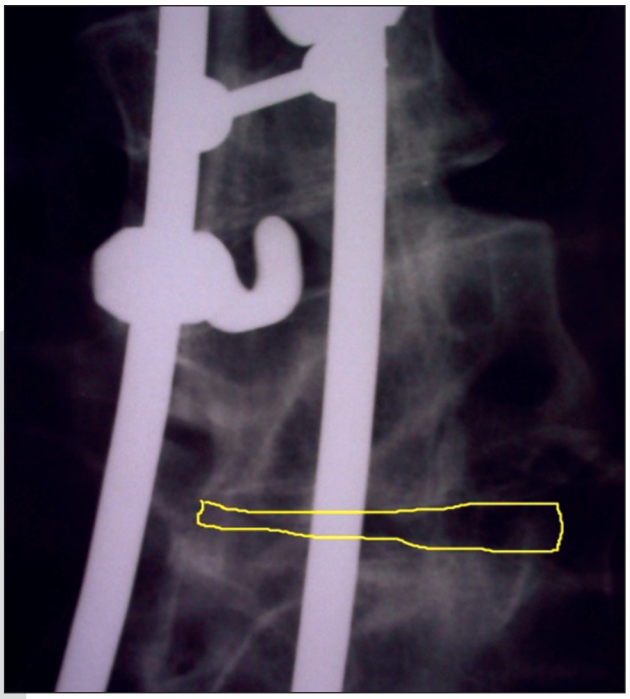

Figure 4. The thickness of intervertebral disc below the apical vertebra after the operation (example)

Relation of the preoperational measures of thickness of the lower apical disc, on the concave side of the curve in all the examined patients with measured thickness after the operation, shows sta-

Table 1. Average thicknes of apical discs preoperatively, postoperatively and at the end of the treatment

Valid N 24		Upper apikal disk (cm)		Lower apical disc (cm)	
		convex side	concave side	convex side	concave side
Preoperatively	*Mean*	0,49	0,25	0,50	0,24
	Minimum	0,20	0,10	0,20	0,10
	Maximum	0,80	0,80	0,80	0,30
	Std.Dev.	0,14	0,13	0,15	0,06
Postoperatively	*Mean*	0,51	0,45	0,49	0,44
	Minimum	0,30	0,20	0,20	0,20
	Maximum	1,00	0,80	0,80	0,80
	Std.Dev.	0,17	0,13	0,16	0,12
At the end of the treatment	*Mean*	0,52	0,46	0,49	0,42
	Minimum	0,30	0,20	0,20	0,20
	Maximum	1.00	0,80	0,80	0,80
	Std.Dev.	0,16	0,13	0,16	0,12

Table 2. Measured values of Cobb's angle.

	Valid N	Mean	Minimum	Maximum	Std.Dev.
Preoperatively	24	53,54167°	33°	97°	15,33674
Postoperatively	24	20,04167°	7°	47°	10,04546
At the end of the treatment	24	20,04167°	7°	47°	10,04546

tistically significant correlation ($r = 0,43$; $p < 0,05$).

The value of the Cobb's angle on the examined radiograms after the operation was in average smaller for 62,57%. The same measured values of Cobb's angle in all the patients remain till the treatment completion (Table 2).

Relation of the measured sizes of Cobb's angle in patients before the operation to those obtained after the operation shows very high factor of correlation ($r = 0,85$; $p < 0,05$), what points to a decrease of angle of the curve for approximately 62,57%, what can be expected in such operations. Christodoulou et al. (2002) in his study recorded an average correction of Cobb's angle on the examined patients with adolescent idiopathic scoliosis than 73% of primary and with curvature, slashed, 59^0 preoperatively to an average of 13^0 postoperatively. At the end of treatment, the mean Cobb's angle was 14^0 . Arlet et al. (2004) have noted in their research far less correction curve, averaged 54% Correction of Cobb's angle for an average of $34,8^0$. Yu et. (2004) operated on the 17 King type II patients. Cobb angle of the thoracic curve before surgery was on average $56,9^0$, $21,6^0$ after surgery, average correction of 60,1%. Kwan Isar. (2004) included the group of 30 patients with adolescent idiopathic scoliosis. Average age of the patients was 16 years. Were followed for 13-34 months. Mean curvature at Cobb's, was 70^0 preoperatively and postoperatively 38^0 or 42^0, which represents 46,7% or 60% correction in a longer follow-up. Our results are similar to those of autors (8,9,10,11).

Conclusion

Wedge-shaped appearance of upper apical disc or different percentage in disc thickness on the concave and convex side of the curve of upper and lower apical disc was decreased after the operation. At the treatment completion insignificant increase (for 0,01cm) in this disc thickness either on concave or convex side of the curve was recorded. Statistical correlation data point to increased percentage (≈90%) in the upper and lower apical disc thickness on the concave side of the curve, and decreased percentage of the curve for approximately 62%, what can be expected with great possibility in such operations.

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Value of P dispersion on elite athletes

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Abstract

Objectives: Our primary objective was to evaluate the valuability of P Dispersion to predict rhytm abnornalities especially atrial fibrillation on elite athletes.

Background: It is mentioned that there is an increased risk for atrial fibrillation in the regular, long term and force requiring exercising young athletes.

Methods: Our study consists of age, sex, BMI and blood pressure measures matched 68 heavy exercising athletes (group 1) and sedantery living healthy individuals. P wave times measured by usage of 12 lead surface ECG. P dispersion (P max-Pmin) was evaluated as the difference between determined maximum and minimum P waves.

Results: Age, BMI (kg/m2), systolic and diastolic blood pressures were the same as in two groups. Heart rates (beat/min.) were significantly lower in group I. ($p<0.05$) When we reviewed echocardiographic parameters there were no significant difference between ejection fractions of two groups. Left ventricule diastolic and systolic diameter (mm), diastolic interventricular septum thickness (mm) and left atrial diameter (mm) were significantly higher in athletes group from control group. Maximum and minimum P wave times and P wave dispersion founded significantly higher in athletes group.

Conclusion: There is an increased risk of atrial fibrillation at athletes who are long time force reguired exercising and who have a dilated left atrium. Especially athletes with left atrial dilatation must be followed up more closely and in these group athletes physical exercise limitations must be re-evaluated

Key words: P dispersion, atrial fibrillation, athletes.

Introduction

Long-time physical activation will make some structural and functional alterations with changing the heart by the hemodynamic alterations. These alterations are known as athlete's heart. Echocardiographic studies showed morphological alterations. These alterations generally contain an increase of left ventricular mass, diameter, wall thickness and left atrial diameter[1, 2]. Although these alterations can be changed with the type of the sport, these are physiological responses for volume and pressure load. Also, with the kind of exercise called dynamic or static; most of the sport branches contain two of these exercise programmes. Sport types like wrestling, weight lifting, body building are static and long distance runnig and basketball are dynamic exercise programs.

According to the density of the exercise programme some electrocardiographic rhythm and conduction abnormalities can be observed in athletes. These abnormalities are sinusal bradycardia, sinusal arrhythmia, atrioventricular blocks, morphological P and QRS alterations repolarisation abnormalities and left atrial dilatation [3, 4].

Nowadays we use a non-invasive technic known as P wave dispersion to predict development of arrhythmia. We can calculate Pwave dispersion with the removal of the minimum P wave time from maximum P wave time on the 12 channel surface ECG. P wave dispersion is a method for predicting atrial fibrillation risk which has a relation with non -homogen and discontinuous transmission of sinusoidal impulses in the intra-atrial and inter-atrial area [5, 6.]

It is known that there is an increased risk for atrial fibrillation in the regular, long term and force requiring exercising young athletes and majority of these athletes were male. Suggested mechanisms are speculative. Atrial ectopic pulses,

inflammatory changings and enlarged atrial diameter are the suggested mechanisms [7, 8].

In this study we aimed to evaluate the P wave time and dispersion with echocardiographic parameters in young male athletes who are undergoing long term regular exercise.

Methods

Patient population

Our study consists of age, sex, BMI and blood pressure measures matched in 68 heavy exercising athletes (group 1) and 25 healthy volunteers who are not exercising regularly. Athletes and individuals of the control group were male. In group 1 there were 25 wrestlers, 24 long distance runners and 19 boxers. In group 1 all of the individuals were undergoing sport over the 4 years from16-20 hours a week. None of the individuals had a cardiac illness and physical examinations were normal. In the group of athletes we excluded the individuals who had interrupted exercise periods over 14 days. We also excluded smokers and alcohol drinkers. All of the individuals were informed about the study and gave informed consent and the study was approved by our hospital's ethic committee.

Electrocardiography and Measurement of P Wave Dispersion

P wave times were measured by usage of 12 lead surface ECG. All of the individuals ' ECGs were recorded at supine position with Nihon Cohden Cardiofax M ECG 1350-K device. Recording speed was standardised at 50 mm/sn and 10 mm/mv. ECG recorded in a silent room after a 20 minute resting period and during recording time we maintained individuals breathing at normal and without talk. The P wave time measurement was made by two cardiologists who were unaware of the clinical situation of the individuals for in order to decrease margin of error, we used a ruler and a magnifying glass. At the end of the study we choosed 10 individuals randomly and corrected the results. The accepted P wave starting point was separated point of P deflection from isoelectric point and accepted P wave ending was intersected point of isoelectric point. P dispersion (P max-Pmin) was evaluated as the difference between the determined maximum and minimum P waves [6].

Transthoracic Echocardiography

All of the individuals' echocardiographic measurements were made at the left lateral decubitis position with Vingmed Cardiac Ultrasound System (Vingmed System 7 General Electric Harten, Norway). Left ventricle end diastolic diameter (LVDD), left ventricle end systolic diameter (LVSD), left atrium diameter, diastolic thickness of interventricular septum and posterior wall (IVSd, PWd) measured with M-mod imaging at parasternal long axis [9]. Right atrium and right ventricle diameter measured at apical four chamber imaging. Left ventricular ejection fraction was measured with the Modified Sympson Method.

Statistical Analysis

Statistical analysis made by SPSS (Scientific Packages for Social Sciences, İnc. Chicago IL, USA) computer programme. All of the data is expressed as +/- standard deviation. The student t test was used to compare data between independent groups. The Bland-Altman analysis was used to evaluate inter and intra-observer variability.

Result

Demographic features of Group I and Group II are presented in Table 1. Age, BMI (kg/m^2), BSA (m^2), systolic and diastolic blood pressures were the same in the two groups. Heart rates (64.8±5.5 vs. 69.4±6.0 beat/min., $p<0.05$)were significantly lower in group I. When we reviewed echocardiographic parameters there were no significant difference between ejection fractions of the two groups. Left ventricle diastolic (48.9±3.9 vs. 45.1±3.8 mm, $p<0.01$) and systolic diameter (33±3.5 vs. 29.4±3.5mm, $p<0.01$)), diastolic interventricular septum thickness (11.5±1. 1vs. 9.3±1.1 mm, $p<0.001$) and left atrial diameter (39.1±3. 1 vs. 36.6±2.8 mm, $p<0.01$) were significantly higher in the athlete group from the control group. Right heart chambers were the same in the two groups. Maximum and minimum P wave times (P max., 132±13 vs. 119±13 ms, $p=0.007$ and Pmin., 92±9 vs. 82±10 ms, $p=0.011$) and P wave dispersion (43±15 vs. 34±13 ms, $p=0.001$) were found to be significantly higher in the athlete group (Table 2). Inter and intraobserver variabilities detected as 5% and 7. 2%.

Table 1. Demographic data of the cases

	Group1 (n=68)	Group2 (n=25)	p
Gender(male)	68	25	-
Age	21±4	22±5	NS
Body mass index (kg/m²)	22. 2±2. 8	23. 1±2. 9	NS
Systolic blood pressure(mmHg)	106. 9±10	104. 5±11	NS
Diastolic blood pressure (mmHg)	66. 6±7. 5	64. 8±6. 2	NS
Heart rate (atim/dk.)	64. 8±5. 5	69. 4±6. 0	<0. 05
EF(%)	62. 9±4. 4	60. 6±4. 9	NS
LVDd(mm)	48. 9±3. 9	45. 1±3. 8	<0. 01
LVSd(mm)	33±3. 5	29. 4±3. 5	<0. 01
IVSd(mm)	11. 5±1. 1	9. 3±1. 1	<0. 001
LA(mm)	39. 1±3. 1	36. 6±2. 8	<0. 01
RA(mm)	35. 3±4. 7	34. 4±5. 1	NS
RV(mm)	35. 1±4. 1	35. 3±5	NS

EF: Ejection Fraction, LVDd: Left Ventricular Diastolic diameter, LVSd: Left Ventricular Systolic diameter, LVPWd: Left Ventricle Posterior Wall Thickness, IVSd: Left Ventricle Diastolic Septum Thickness, LA: Left Atrium, RA: Right Atrium RV: Right Ventricule NS: Statistically Insignificant

Table 2. Comparison of athletes and control groups' P dispersion values

	Group1	Group2	P value
Pmax, ms	132±13	119±13	0, 007
Pmin, ms	92±9	82±10	0, 011
P dispersion, ms	43±15	34±13	0, 001

Pmax: maximum P wave time, Pmin: minimum P wave time

Discussion

Atrial fibrillation and its' everyday increasing incidence is an important etiological factor of mortality and morbidity. Atrial fibrillation has a relationship between hypertension, structural heart disease and hyperthyroidism although we still do not substantially understand its' etiology [10]. In the general population incidence changes between 2-10%. When aetiology is unknown it is defined as lone atrial fibrillation and it is mentioned that there is a tendency towards regular exercise in these patients [11-13]. It has been reported that regular moderate exercise has certain benefits on cardiovascular health and decreases atrial fibrillation risk in adults [14]. Some studies documented the relationship between atrial fibrillation and atrial flutter with long time and intensive exercise [15, 16]. Karjalainen et al. found ten years of atrial fibrillation incidence in intensively exercising athletes 5. 3% and at control group individuals 0. 9% with a prospective study. In this study middle aged intensively exercising athletes' atrial fibrillation incidence were found to be very high [17]. Similarly in Regicor Trial 183 marathon runners and 290 sedentary male individuals were followed for ten years and it was found that yearly lone atrial fibrillation incidence were four times higher in marathon runners (Masia R 1998) Nowadays some studies mention that P wave dispersion is an indicator for atrial fibrillation and atrial flutter for athletes [6, 18]. Our study showed that P wave dispersion of the athlete group was higher than the control group.

Increased diameter of left ventricle and left atrium are expected findings. Scharf et al. showed atrial and ventricular dilatation which are compatible with eccentric and concentric dilatation of the heart by Cardiac MRI [19]. Chen et al. showed that patients which atrial fibrillation have higher atrial diameter when compared with those which without atrial fibrillation [20]. Sharma et al. showed that in elite junior athletes ' left ventricular wall thickness, end diastolic diameter and left atrial diameter were higher than the control group [21]. D'Andrea et al. suggested that athletes' left atrial

volume increase mildly and this was a physiological adaptation for exercise [22]. In our study left ventricular diameters, left ventricular septum thickness and atrial diameters were found to be higher in the athlete group than the control group.

Left atrial dilatation is a strong indicator of atrial fibrillation. It is expressed that if there is no left atrial dilatation in athletes P dispersion can be the same as the control group individuals. Bjornstad et al. specified that there was no left atrial dilatation and none of the cases had atrial fibrillation or flutter in their 15 years follow up study with Norwegian elite athletes [23]. Similarly, another study with elite women basketball players showed that there was no significant increase in the left atrial and left ventricular diameter and the P dispersions were the same as the control group [24].

It is mentioned that there are some possible mechanisms for the relationship between atrial fibrillation and exercise. Atrial ectopia, especially ectopia around the pulmonary vein has a stimulating effect on atrial fibrillation attacks [25]. It is stated that physical activity may increase atrial and ventricular ectopias [26, 27]. Athletes are prone to bradyarrhytmias because of their increased vagal tonus at rest. Vagal tonus affects sinus and atrioventricular nodes and causes bradycardia. Increased vagal tonus is the reason for dispersion in atrial repolarisation [8, 28]. Another possible mechanism is structural changes such as fibrosis and dilatation in long time physical activity engaging athletes' atriums. Frustaci et al. showed some inflammatory structural changes in patients' atriums who had drug resistant atrial fibrillation [28]. Similarly Swanson et al. explained the relationship between systemic inflammation and C-Reactive Protein enhancement and atrial fibrillation in excessive force required exercise. It has been reported that antiinflammatory agents decreasing C-Reactive Protein and cure atrial fibrillation [29].

Restrictions

P wave dispersion measurements were made manually, although we used a ruler and a magnifying glass to reduce the margin of error. Also, the ECG recording speed was 50 mm/sec. The second restriction is the study population which consisted of young and heterogeneous individuals. However, the echocardiographic data was the same. Another limitation of our study was not following up individuals for atrial fibrillation development because it needs long time.

There is an increased risk of atrial fibrillation at athletes who are force required exercising for a long period of time and who have a dilated left atrium. Some possible mechanisms are mentioned, although we do not know the underlying mechanism as yet. For this advanced studies must be carried out. Especially, athletes with left atrial dilatation must be followed up more closely and in these group athletes' physical exercise limitations must be re-evaluated.

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The relationship between serum CEA and Colon Cancer-Specific Antigen-2 in colon cancer patients

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Abstract

Background: Colon cancer-specific antigen-2 (CCSA-2) is an important marker of colon cell malignancies. The function of CCSA-2 in colon cancer is unknown and is an important objective of future studies.

Materials and Methods: To determine the biological and clinicopathological significance of this and another colon cancer marker, we analyzed the levels of serum carcinoembryonic antigen (CEA) and CCSA-2 in 107 patients using the enzyme-linked immunosorbent assay (ELISA).

Results: Serum CEA and CCSA-2 levels were found to be higher in colon cancer patients compared with controls and correlated with poor differentiation, a greater depth of invasion (pT3 and pT4), lymph node metastasis, venous involvement, and TNM stage (pIII and pIV) compared with patients showing.

Conclusion: Like CEA, serum CCSA-2 is a potentially useful clinical marker of colon cancer and metastasis.

Key words: CCSA-2, CEA, colon cancer.

Introduction

Rectal cancer is one of the most common malignant tumors (1)… . Retrospective studies have shown that multivariate predictive models combining existing tumor markers improve cancer detection rates (2,3). These reports have made it possible to use protein detection methods on serum samples and to determine the association of these proteins with disease progression, contributing to the development of many markers with good diagnostic performance. Currently, none of the existing serum markers, such as CEA, can be used individually for screening (3-9).

Carcinoembryonic antigen (CEA) is an extensively used tumor marker for colon cancer (8,9). Preoperative CEA has been evaluated as an independent factor for staging systems in patients with colorectal cancer(10-13).

CEA can be identified and quantified in in colon cancer tissue and normal mucosa (14-16) Some studies have shown that increased CEA levels within the tumor were correlated with advanced stage (11,16,17). In addition, Cosimelli et al.(18) reported that increased CEA levels within a group of colorectal cancer patients was a predictor of local and distant tumor recurrence after surgery independent of other well-know prognostic variables such as stage and histological grade. Serial monitoring of serum CEA to predict recurrence and evaluate the prognosis of colorectal cancer has been suggested (19), but a lack of sensitivity and specificity preclude the use of CEA in the clinic. Approximately 30% of all colorectal cancer recurrences result in increased serum CEA levels (19). Because no single marker is sufficiently predictive, combinations of multiple markers representing different facets of tumor biology will have a better prognostic potential (19). Therefore, new cancer biomarkers should be developed and evaluated to enhance the diagnosis of this disease (19).

One signature of cancer cells is a change in the secretion of some proteins and their circulation in the blood. These changes have been described in the numerous studies of various cancer types, such as breast, bladder, prostate, liver, pancreas, lung, and ovarian cancers.

An important molecule specific for colon cancer is CCSA-2 (20). This protein is associated with colon cancer and is not found in normal adjacent tissue or in colon tissue from patients without cancer (20).

A study conducted in 2007 (21) reported that CCSA-3 and CCSA-4 levels were elevated in colon cancer patients. In addition, serum CCSA-2 levels can aid the serum-based marker detection of colon cancer.

The aim of this study was to investigate the association between clinicopathologic variables in colon cancer patients, CEA levels and colon CCSA-2 levels.

Materials and Methods

Patients underwent a colonoscopy or colon cancer surgery, and all serum samples were collected from consenting patients. This study was conducted with the approval of the Yuzuncu Yil University Medical Faculty Ethical Committee Board.

Specimens from patients were diagnosed histopathologically and staged according to the TNM-International Union Against Cancer classification system. The clinicopathologic characteristics of the patients are described in Table 1. Among 107 colon cancer patients, 39 (36.4%) had a tumor originating in the right colon, 11 (10.2%) in the transverse colon, and 57 (53.2%) in the left colon.

Table 1. The clinicopathologic variables of patients with colon cancer

Variables	Colon cancer patients (n)
Grade	
Well differentiated	26
Moderately differentiated	34
Poorly differentiated	47
Depth of invasion	
pT1	12
pT2	21
pT3	33
pT4	41
Lymph node metastasis	
Absent	29
Present	78
Venous involvement	
Absent	30
Present	77
pTNM stage	
pI	19
pII	21
pIII	28
pIV	39

The following items were used as the inclusion criteria: presence of carcinoma of the large intestine confirmed by histopathological analysis of the extirpated lesion, absence of distant metastases at preoperative examinations and local metastases in the abdominal cavity inventory during the operation, and extirpation of the neoplasm lesion with a curative intention.

Of the 107 patients, 58 (54.2%) were male and 49 (45.7%) were female. Mean age was 57.3 ± 12.5 years (39 to 70 years). None of the patients had received chemotherapy or radiation therapy before surgery, and none of them had a family history of colorectal cancer. All patients were treated by surgical resection. All four stages of colon cancer were represented: stage I (n=19), stage II (n=21), stage III (n=28), and stage IV (n=39).

The control group comprised 40 healthy volunteer subjects (20 women and 20 men, aged 39-58 years). The absence of diseases such as infection and asymptomatic early adenocarcinoma or adenoma was assessed by clinical history, physical examination, routine laboratory tests, including liver and renal function tests, and a colonoscopy.

Serum samples were stored at -80 °C prior to analysis. The preoperative serum levels of CEA were determined by a commercially available immunoassay ELISA kit (Roche Diagnostics). Serum samples for CCSA-2 were plated in duplicate and in a random order in 96-well Nunc Immunoplate Maxisorb plates and incubated overnight at room temperature. On each plate, samples for each of the patient groups were included and distributed across the plates. Samples were aspirated and then blocked with Super Block solution (Pierce). The blocking buffer was removed, and the samples were then washed with 1xTBS supplemented with 0.08% Tween 20. A polyclonal antibody for CCSA-2 was added, and samples were incubated for 2.5 hours at 37° C. The plates were washed with TBST, the secondary goat anti-rabbit peroxidase-conjugated antibody was added, and samples were incubated at 37° C for 2 hours. The plates were washed with TBST and incubated with TMB substrate at 24° C for 1 minute. The plate was analyzed at 645 nm using a plate reader. Inter-assay and intra-assay variability for each assay was determined by plating various concentrations of the CCSA-2 peptide (0-1 mg/ml).

Statistical Analysis

Normality tests for the patient group were conducted with a one-sample Kolmogorov-Smirnov test and histogram-plotted graphs, and the charts and histograms for the control group were assessed by the Shapiro Wilk test. Mean patient age and its standard deviation in the form of a normal distribution as well as the averages of the other variables, their standard deviations, medians and distribution ranges (25th percentile-75th percentile) (IQR) are shown in the form. Patient age between the 2 groups was compared with an independent samples *t* test. Gender differences were evaluated with a chi-squared test. Correlations were analyzed with a Spearman's correlation test.

Results

CEA and CCSA-2 levels were examined in 107 colon cancer patients and 40 healthy volunteers, and the average serum levels of CEA and CCSA-2 levels are shown in Table 2 and Table 3.

We found significantly higher concentrations of CEA and CCSA-2 in the colon cancer patients compared with healthy volunteers ($p<0.0001$). There were no differences between females and males in all parameters ($p>0.05$).

Serum CEA and CCSA-2 levels were significantly higher in total colon cancer patients compared with volunteers. In addition, both protein levels were elevated in patients showing poor differentiation, a greater depth of invasion (pT3 and pT4), the presence of lymph node metastasis, the presence of venous involvement, and a more severe pTNM stage (In the classification of malignant tumors, T describes the size of the tumor, N describes the regional lymph nodes, and M describes distant metastasis) (pTIII and pTIV) compared with patients showing well-differentiated tumors, a lesser degree of invasion (pT1 and pT2),

Table 2. Mean values of serum CEA and CCSA-2 levels in patients with colon cancer and healthy subjects (mean ± SD)

Variables	Colon cancer patients	Controls	p
CEA (ng/ml)	34.9 ± 10.1	2.2 ± 0.3	<0.0001
CCSA-2(mg/ml)	72.5 ± 23.8	2.0 ± 0.4	<0.0001

Table 3. Clinicopathologic variables and serum levels of CEA and colon cancer-specific antigen-2 in patients with colon cancer (mean ± SD)

Variables	CEA	Colon cancer-specific antigen-2
Grade		
Well differentiated	19.2 ± 8.7	48.6 ± 9.1
Moderat ately differentiated	25.1 ± 11.8	54.9 ± 12.3
Poorly differentiated	40.9 ± 9.5	79.4 ± 11.2
Depth of invasion		
pT1	19.0 ± 10.4	33.5 ± 9.0
pT2	24.8 ± 13.9	38.7 ± 19.7
pT3	30.1 ± 18.7	59.2 ± 20.5
pT4	42.8 ± 20.6	81.3 ± 14.9
Lymph node metastasis		
Absent	22.8 ± 11.3	39.1 ± 10.7
Present	39.5 ± 5.2	80.5 ± 12.4
Venous involvement		
Absent	19.4 ± 12.8	27.5 ± 11.7
Present	38.5 ± 9.3	79.0 ± 19.8
PTNM stage		
pI	28.7 ± 5.4	53.9 ± 11.5
pII	30.4 ± 7.7	59.2 ± 9.9
pIII	34.2 ± 10.0	68.4 ± 10.6
pIV	38.0 ± 9.1	80.3

no lymph node metastasis, no venous involvement, and a less severe pTNM stage (pTI and pTII) ($p<0.001$, $p<0.0001$).

We found a significant correlation between serum CEA levels and serum CCSA-2 levels in the patients ($r=0.71$, $p<0.0001$).

Discussion

In this study, we investigated whether CEA and CCSA-2 levels were associated with clinicopathologic variables of colon cancer patients. We demonstrate that the combination of CEA and CCSA-2 shows significant correlations with clinicopathologic variables and could be a potentially useful biomarker. Venous invasion and lymph node metastasis are known to be important clinicopathologic variables for hematogenic recurrence. CEA and CCSA-2 levels in the sera of colon cancer patients may play a major role in metastasis. We found significantly higher concentrations of CEA and CCSA-2 in the colon cancer patients compared with healthy volunteers ($p<0.0001$). In addition, serum CEA and CCSA-2 levels were significantly higher colon cancer patients showing poor differentiation, a greater depth of invasion (pT3 and pT4), the presence of lymph node metastasis, the presence of venous involvement, and a more severe pTNM stage (pTIII and pTIV) compared with patients presenting well-differentiated tumors, a lesser degree of invasion (pT1 and pT2), no lymph node metastasis, no venous involvement, and a less severe pTNM stage (pTI and pTII) ($p<0.001$, $p<0.0001$).

Because of its cytoplasmic distribution, tissue CEA can easily spread to the stroma and join with circulating blood (22,23).

In vitro studies have shown that passing colonic mucosa cells in a calcium-rich culture medium leads to an intracellular build up of CEA (24,25).

Tumor categorization is correlated with CEA levels because tumor cell invasion releases more CEA into the bloodstream (26).

The presence of tumor necrosis as well as perineural and perivascular infiltration affects levels of serum CEA (16,22,27,28). No statistically significant correlation has been found between serum CEA and tissue CEA levels (16,22). Well-differentiated tumors with demonstrable levels of apically dispensed tissue CEA may be associated with reduced levels of serum CEA through secretions that may penetrate the intestinal lumen (16,22). CEA does not normally enter the bloodstream (16,22). However, for tumors with a cytoplasmic dissemination of CEA, it may easily diffuse to the stroma and pass into circulation, resulting in increased serum CEA levels (15).

Our study demonstrates that serum CEA circulation was significantly more frequent in cancer patients with lymph node metastasis and advanced colon cancer lesions. Nazato and his colleagues have found results that are consistent with our results (28).

In addition, we investigated serum levels of CCSA-2 in patients with colon cancer. Previous studies on colon cancer tissues have shown that CCSA-2 was present in t the sera of 85% to 100% of advanced adenomas and 100% of colon cancer samples (29). The release mechanism of this protein from the cell has not yet been discovered, but we theorized that this protein may be secreted into the blood by cellular destruction or apoptosis (30).

The CCSA-2 assays have high sensitivity (97.3%) and specificity (78.4%)(31). In a previous study, CCSA-2 was found to be present in malignant cells(31). In addition, CCSA-2 expression has been associated with advanced cancers and was present in the sera of 80% of patients with invasive cancer (31). Additional studies are needed to assess the relationship between CCSA-2 concentration and clinicopathologic variables of colon cancer patients and to determine whether this protein may be useful as a biomarker for the early detection of colon cancer.

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Antioxidant effect on brain tissue and sciatic nerve of sildenafil: An experimental diabetic rats model

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Abstract

Background and Aim: Oxidative stress is known to play a role in pathogenesis of involvement of both central and peripheral nervous system in diabetes. The aim of this study was to investigate if sildenafil has a protective effect on injury in sciatic nerve and brain tissue of diabetic rats.

Methods: A total of 36 rats was divided into 4 groups as control group (n=9), diabetic group (n=9, only Streptozotocin), diabetic group given sildenafil (n=9, Streptozotocin +), and the group given sildenafil only (n=9). Total oxidant status, oxidative stress index, malondialdehyde, total antioxidant status, and catalase levels were measured to assess oxidative stress in brain and sciatic nerves of the rats.

Results: Total oxidant status, oxidative stress index, malondialdehyde levels in brain and sciatic nerve of diabetic rats were significantly higher compared to controls ($p<0.05$), whereas total antioxidant status and catalase levels were significantly lower ($p<0.05$). Diabetic group given sildenafil had a significantly lower. Total oxidant status, oxidative stress index, malondialdehyde levels ($p<0.05$), and significantly higher total antioxidant status and catalase levels ($p<0.05$) in brain and sciatic nerve compared to diabetic group.

Conclusion: This study showed that sildenafil has a protective effect on the damage in both brain tissue and sciatic nerve of the diabetic rats due to oxidative stress.

Key words: Sildenafil, diabetic, brain, sciatic nevre, oxidative stress.

Introduction

Diabetes mellitus (DM) causes serious harm to nervous system and is the most common condition leading to neuropathy or peripheral neural damage. It also involves central nervous system with a predilection for the white matter (diabetic leukoencephalopathy). In peripheral nervous system, it causes various types neural damage which include diffuse damage (polyneuropathy) and focal damage (mononeuropathy). Oxidative stress plays an important role in the pathogenesis of these complications of DM (1). Oxidative stress arises from an imbalance between free radical production and antioxidant defense mechanism, ultimately leading to tissue injury. Some previous studies have shown that free radical production has detrimental effects on axons, Schwann cells, and probably neuronal perikarya by generating oxidative and nitrergic stress (2,3). Some studies on diabetic rats suggest that some drugs with antioxidant properties can be used for prevention of development of Reactive oxygen species (ROS) and the damage they inflict (4,5). Sildenafil citrate selectively inhibits phosphodiesterase 5 (PDE5), an intracellular enzyme which degrades cGMP. Sildenafil is widely used for erectile dysfunction. In addition to this widely-accepted indication, potential new indications has been arising as it is learnt that PDE-5 is present in various tissues including smooth muscles of vascular and bronchial trees and brain tissue, mostly at cerebellum, hippocampus, and superior cervical ganglion (6-10).

The aim of this study was to investigate if sildenafil has a protective effect on damage in sciatic nerve and brain tissue of streptozotocin-induced diabetic rats.

Materials and methods

Animals

Female Wistar rats (Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals prepared by the Dicle University Animal Ethical

Committee and approval was issued for this study by Dicle University Animal Ethics Committee for 2 years starting from October 2010) weighing 180–250 g were used in this study. The animals were housed under controlled environmental conditions at a temperature of 23 ±2ºC and with a 12-h light/dark cycle. Animals freely accessed to food and water.

Induction of diabetes and treatment protocol

After an overnight fasting period, diabetes in rats was induced with intraperitoneal streptozotocin solution (STZ) at a dose of 45 mg/kg (10). STZ was freshly dissolved in citrate buffer (0.01 mol/L, pH 4.5). Control rats were given buffer only. One week after STZ administration, presence of diabetes was confirmed by measuring a blood glucose value above 250 mg/dl. A total of 36 rats was divided into 4 groups. Group 1, normal control (n = 9), Group 2, diabetic control (STZ, untreated diabetic) (n = 9), Group 3, diabetic + sildenafil treatment (STZ + sildenafil, sildenafil-treated diabetic) (n = 9), Group 4, sildenafil treatment (sildenafil, non-diabetic) (n = 9). Sildenafil was given via orogastric lavage at a dose of 1,5 mg/kg/day for 21 days. After 21-day drug therapy was completed, animals were sacrificed by cervical dislocation, following which brain and sciatic nerve were excised at 4°C and washed with ice-cold saline. The excised tissues were immediately stored at −50°C for biochemical analysis.

Biochemical Analysis

Weight of the excised whole brain and sciatic nerve samples was recorded and samples were taken to storage unit at − 50°C. These tissues were homogenized in five volumes (w/v) of the same solution. Assays were performed on the supernatant of the homogenate prepared at 14,000 rpm for 30 min at+4°C. Lowry's method was used to measure protein concentration of the tissue (11). Cerebral and sciatic tissue lipid peroxidation levels were expressed as malondialdehyde (MDA). MDA was measured as described by Ohkawa et al. (12). The TAS of supernatant fractions was measured with a new, automated, colorimetric measurement method developed by Erel (13). This method was utilized to produce hydroxyl radicals which are the most potent biological radicals.

In the assay, the ferrous ion solution present in reagent 1 is mixed with hydrogen peroxide present in reagent 2. The resulting radicals like brown-colored dianisidinyl radical cations produced by the hydroxyl radicals are also potent radicals. With this method, the antioxidative effect of the sample is measured against the potent-free radical reactions triggered by the hydroxyl radicals produced. The assay has excellent precision values below 3%. The Total antioxidant status (TAS) results are expressed as nmol Trolox equivalent/mg protein.

The Total oxidant status (TOS) of supernatant fractions was assessed with a new, automated, colorimetric measurement method by Erel (14). Oxidants present in the sample oxidize the ferrous ion–o-dianisidine complex to ferric ion. Glycerol molecules enhance the oxidation reaction, which are plentifully present in the reaction medium. A colored complex is formed by the ferric ion with xylenol orange in an acidic medium. Spectrophotometrically-measured color intensity is related to sample's total amount of oxidant molecules. The assay is calibrated with hydrogen peroxide, and the results are expressed in terms of nmol H2O2 equivalent/mg protein (15). The ratio of TOS level to TAS level was accepted as the oxidative stress index (OSI) (16). Cerebral and sciatic tissue TOS and TAS levels were expressed, in respective order, as μmol H2O2 Equiv./gram protein and nmol Trolox equivalent/mg protein.

Catalase activities were measured by assessing the decrease in hydrogen peroxide concentration at 230 nm, as described by Beutler (17).

Statistical Analysis

Data were presented as means ± standard deviation (SD). A computer program (SPSS 11.5, Chicago, IL, USA) was used for statistical analysis. The one-way analysis of variance (ANOVA) and post hoc multiple comparison tests (LSD) were performed on the data of biochemical variables to examine the difference among groups. A p-value of <0.05 was considered as statistically significant.

Results

Levels of TAS, TOS, OSI, MDA, and catalase in brain tissue of rats are given in Table 1. Comparison between group 1 and 2 revealed that diabetic

group had significantly higher TOS (40.1±2.6), OSI (22657±8670), MDA (458.9±61.1) levels (p=0.001), while significantly lower TAS (0.20±0.08) and catalase (0.72±0.14) levels (p=0.001). Comparison of group 2 vs group 3 showed that diabetic+sildenafil group had significantly lower TOS (28.3±5.1), OSI (8402±2442), and MDA (319.0±50.0) levels (p=0.001), and significantly higher TAS (0.35±0.08) (p=0.007) and catalase (0.97±0.11) (p=0.004) levels. Comparison of group 1 and group 4 showed that TAS, TOS, OSI, MDA, and catalase levels were similar (p>0.05).

TAS, TOS, OSI, MDA, and catalase levels in sciatic nerve tissue of rats are given in Table 2. Comparison between group 1 and group 2 revealed that diabetic group had a significantly higher TOS (20.8±1.3), OSI (32937±14377) and MDA (30.5±4.1) levels (p=0.001), whereas a significantly lower TAS (0.07±0.03) and catalase (0.21±0.04) levels (p=0.001). when group 2 and group 3 were compared, diabetic+sildenafil group had significantly lower TOS (14.8±2.9), OSI (11059±3497), and MDA (21.3±3.5) levels (p=0.001) and significantly higher TAS (0.14±0.03) (p=0.001) and catalase (0.30±0.03) (p=0.003) levels. Group 1 and group 4 were similar in terms of TAS, TOS, OSI, MDA, and catalase levels (p>0.05).

Discussion

It has been shown that nonenzymatic glycation, metabolic stress secondary to changes in energy metabolism, sorbitol pathway activity, hypoxia, and tissue damage associated with ischemia-reperfusion injury in diabetes all increase free radical production with resultant decreased antioxidant level (4,18). Increased production of ROS including superoxide, hydrogen peroxide and hydroxyl radical leads to oxidation and modification of structure of cellular proteins, nucleic

Table 1. Biochemical parameters in control (n=9), diabetic (n=9), sildenafil (n=9), and diabetic + sildenafil (n=9) groups in the brain of rats

Groups	MDA (nmol/ gr protein)	TOS (mmol H_2O_2Eq./g protein)	TAS (mmol TroloxEq./g protein)	OSI (H_2O_2/Trolox)	Catalase (U/g protein)
Control (I)	262.7±55.3	22.5±7.2	0.45±0.09	5236±2262	1.21±0.13
Diabetic (II)	458.9±61.1	40.1±2.6	0.20±0.08	22657±8670	0.72±0.14
Diabetic+sildenafil (III)	319.0±50.0	28.3±5.1	0.35±0.08	8402±2442	0.97±0.11
Sildenafil (IV)	264.8±37.9	22.1±7.3	0.50±0.17	5364±3699	1.21±0.25
P values					
I-II	0.001	0.001	0.001	0.001	0.001
II-III	0.001	0.001	0.007	0.001	0.004
I-IV	N.S.	N.S.	N.S.	N.S.	N.S.

N.S; Not significant, MDA; Malondyaldehyde, TOS; Total oxidant status, TAS; Total antioxidant status, OSI; Oxidative stress index.

Table 2. Biochemical parameters control (n=9), diabetic (n=9), sildenafil (n=9), and diabetic + sildenafil (n=9) groups in the sciatic nerve tissue of rats

Groups	MDA (nmol/ gr protein)	TOS (mmol H_2O_2 Eq./g protein)	TAS (mmol Trolox Eq./g protein)	OSI (H_2O_2/Trolox)	Catalase (U/g protein)
Control (I)	17.7±2.9	11.6±3.2	0.17±0.02	6985±2100	0.36±0.02
Diabetic (II)	30.5±4.1	20.8±1.3	0.07±0.03	32937±14377	0.21±0.04
Diabetic+Sildenafil (III)	21.3±3.5	14.8±2.9	0.14±0.03	11059±3497	0.30±0.03
Sildenafil (IV)	17.6±2.5	11.4±3.8	0.18±0.06	7482±5132	0.36±0.08
P values					
I-II	0.001	0.001	0.001	0.001	0.001
II-III	0.001	0.001	0.001	0.001	0.003
I-IV	N.S.	N.S.	N.S.	N.S.	N.S.

N.S; Not significant, MDA; Malondyaldehyde, TOS; Total oxidant status, TAS; Total antioxidant status, OSI; Oxidative stress index.

acids, and membrane lipids. Structural and functional cellular damage leads to cell necrosis and activation of genes involved in neuronal damage (3). Studies in diabetic rats have shown oxidative changes in cerebral cortex and peripheral nerves (19,20). Antioxidants interact with each other in plasma and their collective antioxidant effects are greater than individual antioxidant effect of each single antioxidant. Therefore, TAS measurement is more useful than measuring each antioxidant in determination of oxidant-antioxidant balance (13). Similarly, TOS measurement can be used as an indicator of all free radicals which are formed. In addition, MDA, which is one of the chief end products of lipid peroxidation, is also commonly used to assess oxidative damage. In a study examining the levels of TAS and MDA both prior to and after diabetic treatment of diabetic patients, diabetic patients had a higher MDA level and a lower TAS level; and following antidiabetic therapy, MDA levels decreased while TAS levels increased (5). In our study, we found a significantly higher levels of TOS and MDA levels in both brain and sciatic nerves of diabetic rats compared to controls whereas TAS level was lower. OSI arising from increased oxidative stress was also higher in brain and sciatic nerve of diabetic rats compared to controls. These results of our study are consistent with the literature suggesting the role of oxidative stress in the pathogenesis of brain and sciatic nerve injury developing in DM.

Catalase is an antioxidant enzyme critically involved in protection against detrimental peroxide. Catalase contains heme and converts hydrogen peroxide to water and oxygen, mitigating its toxic effects (4). In a study comparing the antioxidant enzymes in patients with or without diabetic neuropathy (3), patients with diabetic neuropathy had decreased levels of superoxide dismutase, glutathione peroxidase levels whereas TAS and catalase levels were not significantly different. Our study showed that both catalase and TAS levels were significantly lower in brain and sciatic nerve of diabetic rats compared to control group. This low level suggests that oxidative balance changes to the detriment of antioxidant defense mechanism.

Studies showing the role of oxidative stress in development of diabetes-associated complications have suggested that use of drugs with antioxidant properties may be beneficial in prevention of these complications.

Sildenafil is a type-5 phosphodiesterase (PDE-5) inhibitor increasing cGMP levels in response to NO, which augments relaxation of vascular smooth muscle. Protective effect of sildenafil on streptozotocin-induced diabetic rats by its anti-inflammatory and antioxidant effects has been shown (21). In addition, it has been suggested that sildenafil crosses blood brain barrier and has a neuroprotective effect by increasing neurogenesis in an experimental ischemia reperfusion model of middle cerebral artery (22,23). There are experimental proofs that anti lipid-peroxidation properties of either cAMP or cGMP analogs in rat neural (24). In another study which measured TAS and MDA levels in plasma of streptozotocin-induced diabetic rats, phosphodiesterase inhibitors have been shown to have antioxidant properties (9). There are no studies suggesting a protective effect of sildenafil on cerebral damage induced by diabetes. We found that levels of TOS, OSI, and MDA in both brain and sciatic nerve tissues of diabetic rats significantly lowered while TAS and catalase levels increased following sildenafil treatment. These findings suggest a protective effect of sildenafil on cerebral and sciatic tissue damage in diabetic rats induced by oxidative stress.

In conclusion, these results indicated that increased parameters of oxidative stress in the sciatic nerve and brain tissue of diabetic rats indicates that oxidative stress has an important role in the pathogenesis of diabetic neuropathy and encephalopathy. In addition, determination of the protective action of sildenafil on brain and sciatic nerve tissues may suggest the usage of sildenafil in prevention of complications of diabetes.

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Management and treatment of genitourinary traumas in children

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Abstract

Backraund: The aim of this study is to assess and compare the occurence of other organ injuries, management methods, results and complications in patients with genitourinary injuries who presented to our clinic in the last six years with those in the literature.

Material and Methods: The number of patients who were assessed in our clinic due to blunt and penetrating traumas to the genitourinary organs was 87 between Januarys 2004-2011.

Results: Eighty seven patients aged between 0-16 years (mean 6.7 years) were included in the study. 44 (50.5 %) patients were boys and 43 (49.5%) patients were girls. Renal injury occurred in 35 patients in total. Twenty nine patients were managed conservatively and 6 surgically. The ureter was found to be broken in 1 patient and it was repaired by primary repair over a double J stent. Bladder perforations were seen in 5 patients. There was posterior urethral injury in 8 patients; 5 (63%) of them were incomplet and the others were (37%) completely. Thirty female patients had genital traumas.

Conclusion: Genitourinary traumas may occur for several reasons. Conservative therapy may be chosen for hemodynamically stable patients with renal lacerations and most of these types of injuries recover with conservative therapy. Patients should be monitored closely for some time before a decision to go ahead with surgery is taken. Retrograde urethrograms and cystograms should be done for patients with pelvic bone fracture and hematuria and bladder kand urethral injuries should be ruled out.

Key words: Genitoürinary, trauma, children, pediatric, treatment, management.

Introduction

The genitourinary system is frequently affected by traumas in children. The kidneys, ureter and urinary bladder are located in relatively more sheltered parts of the body than the urethra and genital organs [1].

Blunt abdominal trauma is the most common cause of kidney injuries in children. However, penetrating injuries are seen in older children [1,2]. Urinary system organs are injured in 3% of all blunt abdominal traumas. The kidney is the most affected organ in urogenital system injuries. Renal injuries are more commonly seen in children than in adults because of the lower localization of the kidneys in children than in adults, and by an undeveloped Gerato's fascia and flexible 11th and 12th ribs in children [3]. Bladder injury occurs as a result of two mechanisms. The first, in particular, occurs in children more than in adults as a result of blunt trauma to a full bladder located in the abdomen, the other involves penetrating pelvic bone fractures [4]. In girls the urethra is very short and mobile and the pelvic bones are more flexible. Thus, urethra injuries occur less than in boys [5]. In boys the ürethra is longer and more tightly attached to the surrounding tissues; as a result it is more affected by traumas, especially in pelvic bone fractures. The posterior urethra can be injured in traffic accidents and in falls from height. In addition, iatrogenic injuries are common [4,5].

The aim of this study is to assess and compare the occurence of other organ injuries, management methods, results and complications in patients with genitourinary injuries who presented to our clinic in the last six years with those in the literature.

Material and method

The number of patients who were assessed in our clinic due to blunt and penetrating traumas to the genitourinary organs was 87 between Januarys 2004-2011. Forty four (50.5%) patients were boys and 43 (49.5%) were girls. The patients were aged between 1-16 years old (mean age 6.7 years).

Additional pathologies, ages, sex, management modalities, morbidities, mortalities and complications were evaluated. All patients with urogenital injury were included into the study. Patients with abdominal traumas whose vital signs were stable underwent ultrasonography (US) and computed tomography (CT).

Patients with renal injury were admitted to the intensive care unit and their vital signs (heart rate, respiration rate, blood pressure, amount and density of urine) were monitored hourly and their hemoglobin levels at 6 and 24 hours. History, physical examination, US and/or CT were used for diagnosis. Need for blood transfusion and management methods were judged. Patients who were hemodynamically stable were managed conservatively. Patients who had signs of acute abdominal trauma and falling hemoglobin levels in spite of blood transfusions underwent surgery.

Injured bladders were repaired surgically and patients were managed with a urethral Foley catheter for 4-5 days. Patients with a suspected posterior urethral injury were screened with urethrogram. A cystodrain catheter was fitted in patients with posterior urethral injury and these patients were followed up for three weeks. A urethrogram screening was performed for these patients after this period and those who did not improve underwent surgery.

Girls with genital lacerations were examined under general anesthesia. The perineum, vagina, hymen, minor and major labias and anorectum were examined. Open sores were repaired with 4/0 polyglactin sutures. Patients who had hematoma, edema, or ecchymosis with no open sores were fitted with a urethral catheter. Penile and scrotal lacerations were repaired primarily in boys. Penile repair was done over an urethral catheter in patients with penile amputation.

The records of patients were evaluated retrospectively. General information, age, sex, mechanisms of traumas and management methods were recorded. Hemodynamic values were assessed with first blood pressures and hemoglobin values and the need for blood transfusion in the follow up period.

Table 1. Evaluation of management methods, additional pathologies, complications and results of patients with genitourinary trauma

	Kidney	Ureter	Bladder perforation	Posterior uretral injury	Genital Laceration (female)	Genital Laceration (male)	Total
	35	1	5	8	30	8	87
Type of trauma (blunt/penetrating)	34/1 (97%/3%)	1	3/2 (60%/30%)	6/2(75%/25%)	20/10(66%/33%)	6/2(75%/25%)	69/18(79%/21%)
Sex (M/F)	23/12(66%/4%)	E	4/1(80%/20%)	8/0	0/30	8/0	44/43(51%/49%)
Censervative management	29(83%)	-	0	0	5(17%)	0	34(39%)
Surgical management	6(17%)	1	5(100%)	8(100%)	25(83%)	8(100%)	53(61%)
Blood transfusion	13(37%)	0	1(20%)	2(25%)	3(10%)	1(13%)	20(23%)
Additional patholagies	16(46%)	1	4(80%)	5(63%)	5(17%)	3(38%)	34(39%)
Camplications	9 (25%)	-	1(20%)	4(50%)	2(7%)	3(38%)	19(22)
Mortality	1(3%)	0	0	0	0	0	1(1%)

Results

Eighty seven patients aged between 0-16 years (mean 6.7 years) were included in the study. 44 (50.5 %) patients were boys and 43 (49.5%) patients were girls.

Renal injury occurred in 35 patients in total, due to blunt trauma in 34 patients and to penetrating trauma in 1. Twenty nine patients were managed conservatively and 6 surgically. Thirteen patients whose hemoglobin levels fell below 10 mg/dL had blood transfusions. There were cases of isolated renal injury in 19 patients, and additional liver or spleen injury in 16. Resorption fever was seen in 3 (9%) patients, temporary hydronephrosis in 2 (5.7%), pneumonia in 1 (2.8%), atelectasis in 1 (2.8%), blood reaction in 1 (2.8%) and hypertension in 1 (2.8%). The total complication rate was 25% (9 patients) for renal injuries.

The ureter was found to be broken in 1 patient and it was repaired by primary repair over a double J stent. There was no complication in this patient.

Bladder perforations (Figure 2A, B) were seen in 5 patients, as a result of blunt trauma in 3 (60%) patients and from penetrating trauma in 2 (40%). The bladder was repaired primarily for each patient. Three patients (60%) had pelvic fracture with bladder perforation, and 1 (20%) had rectal perforation. There was abdominal tenderness in all of the 5 patients and 4 (80%) had hematuria. One patient (29%) had a urinary tract infection.

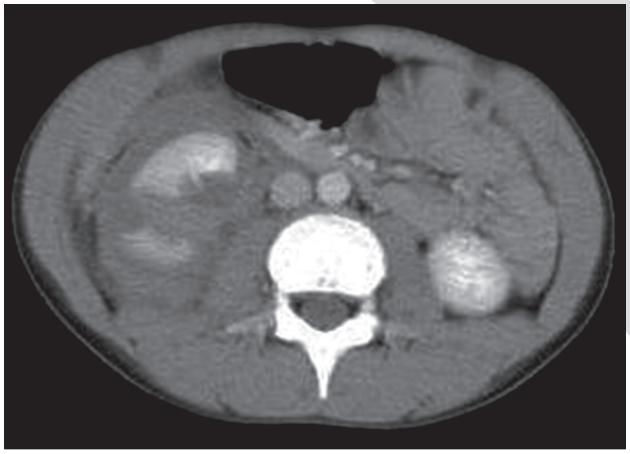

Figure 1. Axial enhanced CT image shows grade 4 renal laceration.

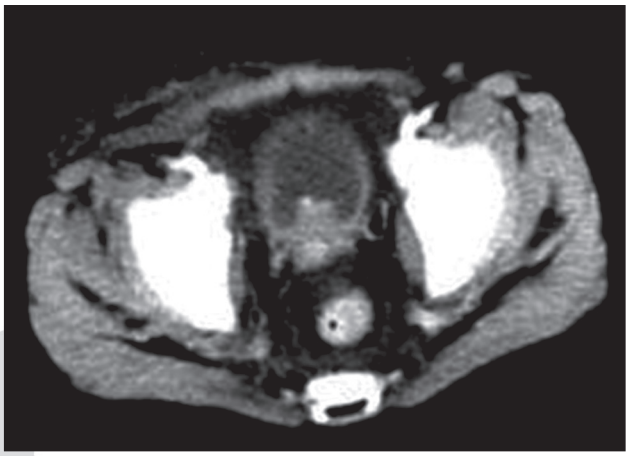

Figure 2 A, B. Bladder perforation

There was posterior urethral injury in 8 patients; 5 (63%) of them were incompletel (Figure 3A) and the others were (37%) completel (Figure 3B). These urethral injuries were diagnosed by retrograde urethrograms. Five of these patients (62.5%) had pelvic fracture and 1 (13%) had bladder injury. A cystodrain catheter was inserted into the bladder and expected for at least three weeks. Urethral injuries recovered in 2 patients (25%) and there was no need for any additional surgery. Late urethroplasty was performed for the other 6 patients (75%). Urethral stricture developed in 3 patients (38%) and incontinence in 1 (13%).

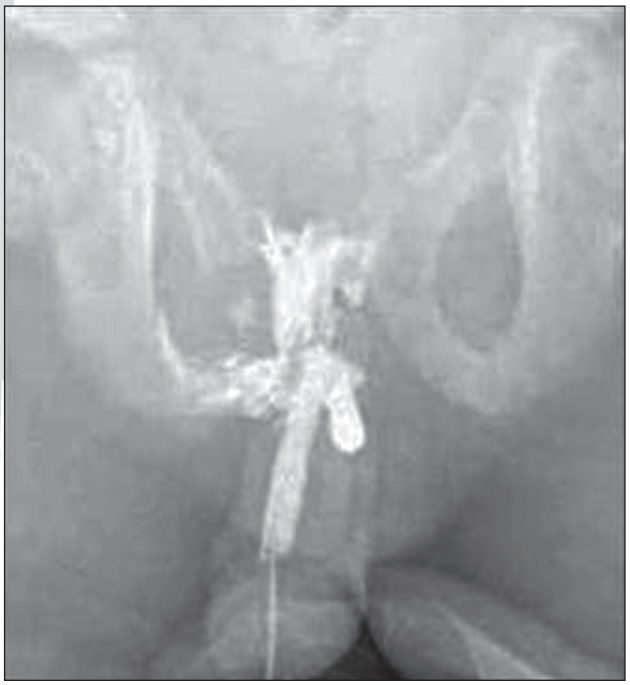

Figure 3A. Incomplete posterior urethral injuries

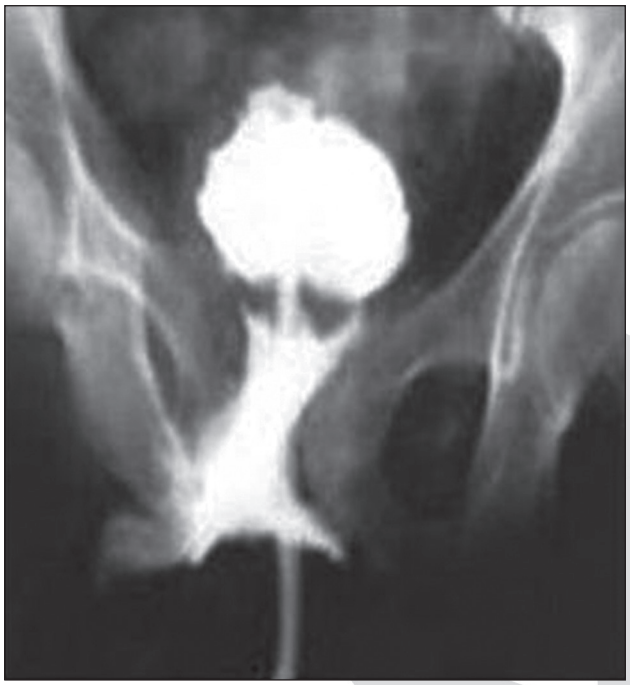

Figure 3B. Complete posterior urethral injuries

Thirty female patients had genital traumas. Eighteen (60%) had blunt trauma, 10 (33%) had penetrating trauma and 2 (7%) had genital lacerations due to sexual abuse. Five patients (17%) had only edema and hematoma and were managed conservatively. The others (83%) had primary repair under general anesthesia. Patients were discharged after follow-up with a urinary catheter for 2-3 days. Wound infection developed in 2 (7%) patients.

There were cases of penile incision in 3 (38%) patients and scrotal incision in 3 (38%) in a total of eight male patients. Two patients who applied because of penile amputation were managed surgically and penile repair was carried out. However, the distal part had become necrotic and this part was excised.

Discussion

Renal injuries are the most commonly encountered injuries due to urinary system traumas and occur in 30-70% of genitourinary injuries. The bladder is injured by blunt trauma in children especially when it is also found full of urine and it is also found together with pelvic fractures [3,4]. Bladder ruptures are seen with pelvic fractures in 75-95% of cases, whereas 4-20% of pelvic fractures occur with bladder rupture. Isolated urethral injury does not threaten life but impotence, stricture and incontinence may occur in the late period. Urethral injury develops with symphisis pubis fractures after blunt trauma. Of children who have pelvic fracture 5% also have posterior urethral injury [3,6-9].

Nance et al. [11] evaluated a total of 95 patients with renal trauma and treated 90 children (95%) conservatively. Mohamed et al. [12] reported that 24 of 37 children (65%) with high grade renal injury were managed conservatively and the others (35%) surgically. In our study, we treated 29 (83%) patients with conservative therapy and 6 (17%) patients with surgical therapy. Patients with high grade renal injury were studied in the Mohamed et al. report, and the rate of conservative therapy was lower than in our study. Patients who were treated surgically in our study had grade 4 or 5 renal injury.

Hypertension, arteriovenous fistula and hydronephrosis are late complications whereas abscess, fistula, infection, urinoma, hydronephrosis, and blood reaction can be seen in the early period [12, 13]. Resorbtion fever was seen in 3 (9%) patients, transient hydronephrosis in 2 (5.7%), pneumonia in 1 (2.8%), atelectasis in 1 (2.8%), blood reaction in 1 (2.8%) and hypertension in 1 (2.8%) patient in this study; so complications were seen in a total of 9 patients (25%).

Between 70-90% of bladder injuries occur as a result of blunt traumas. Pelvic fractures in 70% of bladder injuries. Previous reports show that 5-10% of patients have bladder injury with pelvic fracture [14,15]. Kılıcarslan et al. [16] reported that 3 of 4 (75%) patients with bladder injury also had pelvic fracture. Dokucu et al. [17] evaluated 154 patients with urogenital trauma. Eleven of these patients (7.1%) had bladder trauma, 2 had bladder and urethra injury. They reported that bladder perforations are frequently found in relationship with intestinal perforations. Five patients had bladder perforation among the 87 patients in our study; the cause of bladder perforation was blunt trauma in 3 cases. All of these patients had abdominal tenderness. Hematuria was seen in 4 patients. Three patients had pelvic fracture beside bladder injury. Bladder perforation must not be forgotten in patients with trauma and abdominal tenderness, hematuria and pelvic fracture and diagnostic tools must be used for this condition. İntestinal and uterine perforations must be examined carefully in patients with bladder perforations due to penetrating traumas.

Posterior urethral injuries occur, inparticular, with the crushing or compression of fractured pelvic bones. Anterior urethral injuries are very rare and pelvic bone fractures can accompany anterior urethral injuries [18]. Nerli et al. [19] reported pelvic fracture in 15 of 22 patients (68%) with posterior urethral trauma. Avanoglu et al. [7] reported that there were stricture at rate of 29% and incontinence at rate of 7%- after surgery for posterior urethral injury in 14 patients. Kilicaslan et al. [16] evaluated their 13 patients with posterior urethral injury by means of urethrogram and reported that 8 patients (62%) had complete and 5 patients (38%) had incomplete injuries. They managed 8 patients with urethral repair after two months with cystostomy and 4 patients with early urethral repair. Stricture occurred in 4 patients in the one year follow-up period. In our study, there were urethral injuries due to blunt trauma in 8 patients. All of these injuries were at the posterior urethral level, and were diagnosed by retrograde urethrograms. Five patients (62,5%) had pelvic bone fracture. One patients had a bladder injury beside urethral injury. Incomplere injuries were ameliorated only with suprapubic diversion in two patients (25%). Late urethroplasty was performed in 6 patients (75%) and 1 of these patients underwent primary repair. Stricture occured in 3 patients and incontinence in 1. The results in our patients were compatible to those in the literature. With regard to the rate of urethral injury with pelvic fracture. Also, our rate of 62.5% for pelvic fracture was similar to that in the literature. According to our experiences, urethrogram must be performed immediately in patients who have pelvic bone fracture, bleeding from the urethral meatus and cannot micturate. Otherwise, an incomplete injury may become a complete injury. Patients who have genitourinary trauma and pelvic bone fracture must be evaluated with retrograde urethrogram and cystogram and suprapubic diversion must be performed in the early period.

The most common causes of genital traumas are traffic accidents, falls from height, bicycle accidents, etc. Vaginal, urethral, labial, perineal body and rectal injuries together can occur or separately. Damaged tissues must be debrided and repaired with absorbable sutures [20,21,22]. Iqbal et al. [23] reported that they managed 13 patients with simple surgical repair and 7 patients with rectal or perineal reconstruction. They evaluated 7 patients with vaginoscopy and cystoscopy and 4 with rectoscopy and found pathologies at vaginoscopy in 2 patients and no pathologies in rectoscopy and cystoscopy. Genital lacerations were found in 30 patients duo to blunt (18 patients, 60%) and penetrating (10 patients, 33%) traumas and sexual abuse (2 patients, 7%). Five patients had genital hematoma and edema and were managed conservatively. Fifteen of the other 25 patients (60%) were treated by simple surgery and 10 (40%) patients by perineal or rectal reconstruction. A colostomy was done in 1 patient due to a laceration extending to the rectum. The hymens of two patients were not intact and hymenoplasty was performed. Two patients had wound infections and these infections healed with antibiotherapy. One had a urinary tract infection due to a foley catheter. Vaginoscopy was performed in 2 patients, cystoscopy in 2 and rectoscopy to 1. There was a foreign body in the vagina of 1 patient. There were no permanent complications in any of the patients.

Conclusion

Genitourinary traumas may occur for several reasons. The possibility of genitourinary injuries must be bone in mind after traumas to the abdomen, pelvis and perineum. Physical examination and advanced imaging technigues should be fonducted carefully. Conservative therapy may be chosen for hemodynamically stable patients with renal lacerations and most of these types of injuries recover with conservative therapy. It has some advantages like short hospitalization time, less need for blood transfusion and decreased morbidity and mortality. Patients should be monitored closely for some time before a decision to go ahead with surgery is taken. Retrograde urethrograms and cystograms should be done for patients with pelvic bone fracture and hematuria and bladder and urethral injuries should be ruled out. Vaginoscopy, rectoscopy or cystoscopy should be done if necessary, otherwise other pathologies or foreign bodies may be overlooked.

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Bispectral index for management of hypnosis anaesthetic goals

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Abstract

Introduction: Depth of anesthesia correlates with hypnotic drug concentrations in CNS, which allows controlling and adjusting the depth of anesthesia by the concentration of the drug. Technique of Bispectral Index (BIS monitoring) is based on the online analysis of cortical EEG which developed and spread in clinical practice. It is used to detect too light and too deep anesthesia.

Methods: This is a prospective, comparative, randomized clinical study. Bispectral index was used for the assessment of anesthesia depth and comparison of general intravenous and general inhalational anesthesia in 100 patients underwent elective abdominal surgery.

Results: The study showed a significant difference in time to achieve optimal BIS value during induction to anesthesia, awakening from anesthesia and speed of changes between in the depth of anesthesia between general anesthesia with Sevoflurane inhalation and intravenous Propofol.

Conclusion: Applied BIS monitoring is used for an adequate assessment of the anesthesia depth and allows adequate use of anesthetics, with the prevention of unintended intraoperative awareness. The results regarding the optimality of general anesthesia preferred inhalational anesthesia with Sevoflurane.

Key words: Anesthesia depth, BIS monitoring, inhalation anesthetic, Sevoflurane, intravenous anesthetic, Propofol.

Introduction

Understanding of anesthesia, as a spectrum of separate pharmacological actions towards different goals that anesthesia achieve, it is almost impossible to determine the depth of anesthesia using only one measuring method[1,2,3]. Depth of anesthesia was defined as a continuum or progressive central nervous system depression with decreased response to stimulus[4]. Specifically defined stimuli and specific responses are needed to define the anesthesia depth [5].

In case of fully awake patients, the function of the central nervous system (CNS) can be tested directly by physical examination. When using a general anesthesia, direct testing of the CNS function is not possible[6]. Monitoring the effects of general anesthesia on the CNS in a quantitative, reliable manner is an important issue for anesthesiologists and objectively measure of the hypnotic component of anesthesia was discussed for decades [7].

BIS monitoring technique (Bispectral Index) is based on the analysis of cortical EEG which enabled the monitoring anesthesia depth and management of anesthetic hypnosis goals, analgesia and immobility by clarifying the difference between the responses of the brain and spinal cord.

BIS value is described using arbitrary numerical units which are ranked from 100 (fully awake) to 0 (absence of brain activity, isoelectric EEG), while the recommended values for surgical anesthesia are 40 – 60. Like other monitors of the hypnosis, BIS measure EEG effects and not the concentration of certain drugs. It shows a good statistical correlation with the loss and return of consciences [8]. (Figure 1)

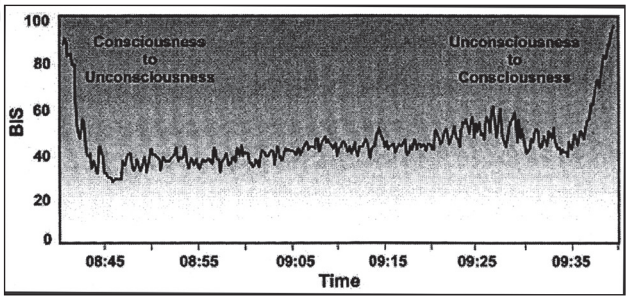

Figure 1. Bispectral index curve

After administration of the anesthetic starts decline in BIS index from 100 in the wake state. Point when individual patients lose consciousness, occurs in a wide range of BIS value [9]. Most often, the loss of consciousness occurs in the range 70 – 80. With the decrease in the BIS value under 70, the possibility of explicit recall is lower. If the BIS value is in the range from 60-40 possibility of intraoperative awareness is extremely small [10]. BIS value below 40 indicates a deep hypnosis. Loss of consciousness in anesthesia and depth of anesthesia correlates with hypnotic drug concentrations in CNS, which allows controlling and adjusting the depth of anesthesia by control of drug concentration. Too deep anesthesia can cause extremely poor postoperative results. On the other hand, light anesthesia creates the conditions for the occurrence of intraoperative awareness which can leave long-lasting effects on the mental status of the patient.

Material and methods

This was a prospective, clinical, comparative, randomized study s performed in 2010 at the Clinical Center of Sarajevo University. The study included 100 patients, aged 30-60 years who were admitted at the Clinic for abdominal surgery for elective surgery under general anesthesia. General anesthesia was performed with Sevoflurane inhalant technique (induction and maintenance of anesthesia) or using intravenous propofol (induction and maintenance of anesthesia) for up to 60 minutes. All subjects were classified as ASA (American Society of Anesthesiologists) I and II group who after determining eligibility were divided into two comparable groups according to the type of anesthesia, with stratification within the groups by sex and age groups as follows: Group S - 50 patients and Group P - 50 patients.

BIS XP system was used for assessing depth of anesthesia. Before induction, to all patients on the forehead was placed unilateral electrode of BIS sensors that recorded EEG waves. The forehead was cleaned with alcohol and dried. Over BIS sensors, connected to the corresponding cable to BIS monitor, EEG recording is converted into a numeric value of BIS index that was read in the upper left corner of the BIS monitor, showing the state of the patient's awareness. Loss of consciousness was defined as loss of eyelash reflex and no response to verbal commands. Return to consciousness is defined by opening the eyes and executing commands. Observed value of the BIS, which shows either light or too deep anesthesia, was corrected by changing the dosage of the drug to achieve the recommended anesthetic value of BIS.

Patients of group P, before the loss of consciousness is administered opioid - Fentanyl (2.5 mg/kg). Oxygenation lasted two minutes before the start of induction. For induction of anesthesia is coordinated by Propofol intravenously as a bolus dose (2.5-3 mg/kg). After loss of consciousness was administered muscle relaxant Tracrium at a dose of 0.5 mg/kg, and oxygenation was continued by manual ventilation until intubation, which was performed three minutes after the start of induction. After intubation, controlled ventilation was established through anesthesiological machine with a ratio of O_2 and N_2O - 1: 1. Anesthesia was maintained by Propofol intravenous perfusion at a dose of 80-120 mg/kg/min. Administration of Propofol and addition of nitric oxide is interrupted with the last skin suture. For interruption muscle relaxation was used Neostigmine with Atropine.

Patients of group S, before the loss of consciousness is administered opioid - Fentanyl (2.5 mg/kg). Oxygenation lasted two minutes before induction. Then the oxygenation was interrupted via the anesthesia machine. The flow of gases to the appliance was staged and the value of 10 L/min, and vaporizer concentration of Sevoflurane was determined at 8 vol%. End of the hose system for anesthesia whith a mask was closed. When the display machine showed that the inspiratory concentration of Sevoflurane reached values close to 8vol%, the mask was quickly placed on the face of the patient and the patient was asked to breathe deeply. Then the flow of gas (100% oxygen) was returned back to 6 L/min and the concentration of Sevoflurane on Vaporizer after every two breaths reduced to 2 vol%. The maximum concentration of Sevoflurane was maintained at 3 vol% and this concentration is used as a concentration for induction of anesthesia with inhaled anesthetic. By loss of consciousness began manual ventilation with a mixture of oxygen and Sevoflurane (3-1 vol%), and was administered a muscle relaxant Tracrium (0.5 mg/kg). Manual ventilation continued until

intubation, three minutes after the start of induction. Maintenance of anesthesia was by inhalation of Sevoflurane in a dose from 1 to 1.5 vol%, in a mixture of O_2 and N_2O 1: 1. Administration of Sevoflurane and N_2O stopped with the last suture the skin. For interruption muscle relaxation was used neostigmine and atropine.

In this study were monitored and recorded:

- The BIS value
- Concentration of Sevoflurane during the introduction (group S)
- Concentration of Sevoflurane during maintenance of anesthesia (group S)
- Dose of propofol during the induction (group P)
- Dose of propofol during maintenance of anesthesia (group P)

As follows:

- Prior to the introduction
- At a loss of consciousness
- Every 10 minutes from the start of anesthesia
- At the awakening from anesthesia

This is a non-invasive method of monitoring which was used in the ordinary course of patient monitoring during the general anesthesia. Registered and monitored parameters in the study are shown in tables and figures, using descriptive statistics by the absolute number of cases, percentage, mean, standard deviation, standard error of the mean and range. For the analysis of statistical significance of differences between the groups is used Student's test and chi - square test depending on the data type. All tests were evaluated with a confidence level of 95% or the value of $p<0.05$ were considered statistically significant.

Results

Of the 100 patients analyzed in both groups there were 25 female respondents (50%) and the same number of male respondents (50%). The total average age was 46±10.62 years. The average value of the BIS index before induction in both groups was 97.

The average time loss of consciousness showed a significant difference between the groups. (36 vs. 46 sec). In the group S patients 10 seconds before lose consciousness than patients in group P (Table 1). There were no significant differences in the time of achieving BIS 40 between the groups. The mean BIS index values was significant difference between groups (group S vs. group P), at a loss of consciousness - t_1 (53 vs. 65), at the 40 minute of surgery - t_7 (47 vs. 51) and the awakening from anesthesia - t_8 (85 vs. 88).

There is a significant difference between groups in the number of patients who had a response to endotracheal intubation. In group P, the percentage of responses is 4% - one respondent. In group S, the percentage is 24%, a total of 12 patients.

There were no significant differences in the mean BIS index values during maintenance of anesthesia (Figure 2). Group P - 47, a group S – 48. These values corresponded to the rank of surgical anesthesia. Total percentage of change from stage of deep anesthesia to surgical anesthesia was 19%. Percentage change from stage of light anesthesia to surgical anesthesia was also 19%. Time, in seconds, which was necessary to change the depth of anesthesia in group P was significantly higher for the change from stage of deep anesthesia to stage of surgical anesthesia and to change from the stage of light anesthesia to stage of surgical anesthesia: 368 vs. 40 and 145 vs. 43 (Table 2, Figure 3)

No significant differences in BIS index values for the stage of deep anesthesia between groups P and S group (27 vs. 28) is noted. For the stage of light anesthesia there is a significant difference between group P and S group (68 vs. 72).

Concentration of Sevoflurane (F_i, MAC and F_e) used in this study correspond to recommended concentrations for maintenance of anesthesia with the use of N_2O. The recommended concentration of propofol for maintenance of anesthesia were 100 -150 μg/kg/min. In this study, an adequate depth of anesthesia was maintained with propofol concentrations in a dose of 81-87 mg/kg/min.

Indications of light anesthesia were corrected by administration of general anesthetics bolus. To the group P was additionally administered 30 mg of propofol, and in group S, F_i of Sevoflurane was increased to 4 vol% with an increase in the flow of fresh gases. Indicators of deep anesthesia were corrected by reducing the concentration of general anesthetics. In group P delivery of propofol via perfusion was reduced to 20 mg/kg/min., and in the group S

was decreased F_i Sevoflurane to 0.5 vol% on the evaporator. The time required for changing stages of anesthesia was significantly shorter in group S.

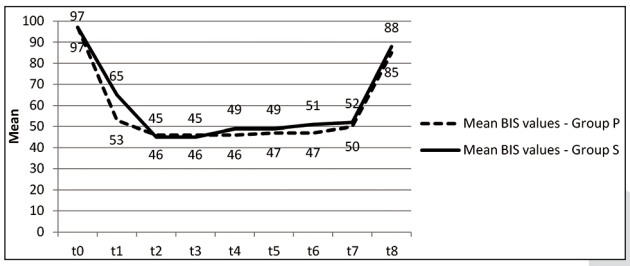

Figure 2. Mean BIS value

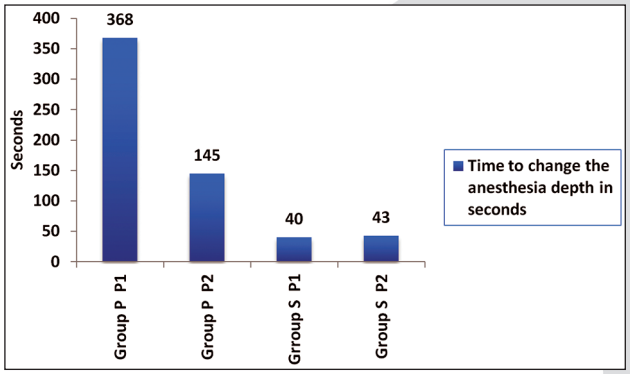

Figure 3. Mean time to change the depth of anesthesia in seconds

Time needed for return of consciousness after cessation of anesthetic dosing was significantly shoter in group S. In group P mean time was six minutes and in the group S the mean time was three minutes.

An interview was carried out with patients from both groups after 24 hours. There were no memories of events during surgery. The incidence of awareness in this study, therefore, was 0%.

Discussion

Anesthesiologist task is to achieve and maintain adequate anesthesia during surgical procedures. The basic mechanism of general anesthesia suggests that there is one component of anesthesia that can be used to define the overall „depth“, but for the anesthesiologist, unconsciousness of the patient and prevention of memory formation during surgery remains a key objective[11]. Depth of anesthesia was and still is most often assessed individually by some anesthesiologists direct by observation of patients and unsophisticated physiological parameters. Poor cardiovascular effects and somatic reflexes (patient movement) are commonly used to estimate the depth of anesthesia.

In clinical practice, light anesthesia may be unrecognized when the patient is relaxed (paralyzed), when it is so weak to move, when the

Table 1. Mean time to loss of consciousness in seconds

The average time until loss of consciousness in seconds						
	N	**Mean**	**Std. deviation**	**Std. error**	**Minimum**	**Maximum**
Group P	50	46	12.4317	1.7581	30.0	100.0
Group S	50	36	11.1991	1.5838	13.0	60.0
Total	100	41	12.7370	1.2737	13.0	100.0

t=16.734 p<0.05

Table 2. The percentage of change in the depth of anesthesia during surgery

			Group P	**Group S**	**Total**
Type of change	P1	N	12	7	19
		%	24.0	14.0	19.0
	P2	N	6	13	19
		%	12.0	26.0	19.0
	No change	N	32	30	62
		%	64.0	60.0	62.0
Total		N	50	50	100
%			100,0	100.0	100.0	

$\chi 2=3.959$ p=0.138

P1 - Change from deep to surgical anesthesia

P2 - Change from shallow to surgical anesthesia

anesthesiologist does not pay sufficient attention to clinical signs or when the time for depth of anesthesia is overtaken by concerns as bleeding, technical problems (empty syringe or Vaporizer), or by in insufficient pharmacological knowledge and inappropriate correction doses. Paralytic agents cause muscle paralysis and often interfere with functional or autonomic nervous system. The patients cannot signal their distress and cannot show signs of consciousness, which are expected to be discovered by clinical signs.

Patient movement in response to surgical stimulation remains an important sign of inadequate depth of anesthesia, but it is uncertain and suppressed by patient relaxation[12]. Light anesthesia occurs as a result subdosage during anesthesia. Subdosage may arise from ignorance or anesthesiologist inexperience, or targeted subdosage due anesthesiology request for an operation (Caesarean section, hypovolemic patients, and patients with minimal cardiac reserve). Smaller doses can also be administered due to the reduction of costs [13]. Traditional clinical signs such as hypertension, tachycardia and tearing are unreliable indicators for the anesthesia depth [14, 15].

Excessively deep anesthesia is difficult to be diagnosed clinically, because it looks like an adequate anesthesia which is characterized by the absence of awareness and response to commands. Usually is recognized when the unwanted effects of the drug are noticed, such as hypotension, bradycardia, prolonged apnea, delayed recovery, etc., which occur at concentrations of anesthetics that are far above the minimum sufficient concentration.

Concentrations of drugs for the same effect vary individually and depend on the age, physiological status, other administered drugs, the intensity of stimulation, etc. The same concentration of the drug may result in unsatisfactory depth of anesthesia in one patient and excessive depth in the second. Anesthesia often starts by delivering doses based on the statistical findings (e.g., dose has a high probability of being adequate), but always should be followed the individual assessment of anesthesia depth in order to customize the delivery of the drug. Anesthesia is a balance between the amount of anesthetic drug(s) administered and the patient's state of awareness. The intensity of stimulation varies during surgical operations, and hemodynamic effects of anesthetics may limit the amount that can be given safely, so it is not uncommon to report a critical imbalance between demands and administration of anesthetic drug. Subdosage or overdose can occur due to inadequate titration of the hypnotic component and this leads to a shallow or too deep anesthesia, which may compromise the patient outcome [13, 16].

Changing the concentration of general anesthetics (inhalational or intravenous) can affect the change in of depth of anesthesia. Electrophysiological monitoring techniques of the brain cortex, helps to titrated delivery of drugs according to individual requirements. This allows quantitative evaluation, which is much more sensitive than clinical assessment. BIS technology may provide new knowledge about the direct and specific effects of anesthetics on the patient's brain. BIS monitoring enables to assess awareness separately from cardiovascular reactivity and to titrate anesthesia according to the needs of each patient. There is a correlation between the concentration of anesthetic (propofol, halogen), clinical signs, EEG effects and the value of BIS index [17]. For propofol and halogone, there are relatively small changes in the index values in relation to the significant changes of concentrations[18,19]. Studies suggests that the incidence of intraoperative awareness during intravenous are higher than during inhalational anesthesia, and the problem is especially pronounced in high-dose opioid anesthesia. It is difficult to assess the real difference in the incidence of intraoperative awareness during intravenous and inhalational anesthesia because statistical calculations show that one such study will require approximately 24000 patients in both groups [20].

In clinical practice to assess the depth of anesthesia is used hemodynamic response to laryngoscopy, endotracheal intubation and skin incision. Reaction to endotracheal intubation is considered autonomous reflex response. Laryngoscopy and endotracheal intubation among the harmful stimulation and are an important reason for intraoperative awareness [16,21]. BIS value is late in responding to this harmful stimulus and this delay is about 45 seconds. Administration of fentanyl and lidocaine spray reduces the hemodynamic response to endotracheal intubation. The effect of fentanyl is more pronounced compared to lidocaine[22].

Conclusion

Applied BIS monitoring is used for an adequate assessment of the anesthesia depth. The advantage of BIS monitoring and the ability to adequately monitor anesthesia depth results in adequate anesthetics use and prevention of unintended intraoperative awareness. The results regarding the optimality of general anesthesia is in favor of inhalational anesthesia with Sevoflurane compared to anesthesia with intravenous anesthetic propofol in terms of more rapid loss of consciousness, more rapid awakening from anesthesia, a shorter period of time during the transition from lighter anesthesia in surgical anesthesia and deeper anesthesia in surgical anesthesia.

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Relationship between serum thyroid hormone and blood lipid in mental disorder patients

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Abstract

Aim: To study the relationship between serum thyroid hormone and blood lipid in mental disorder patients during hospitalization.

Methods: The genders, ages, diagnoses, drugs, doses and hospitalization stays of the 216 included patients were investigated. The levels of fasting serum thyrotropin, thyroid hormone, triidothyronine, free triidothyronine, free thyroid hormone, blood lipids (high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), total cholesterol and triglyceride) and fasting blood glucose, and the blood pressures within 6 weeks were monitored and compared.

Results: The serum triidothyronine levels of male patients were significantly higher than those of female patients ($P<0.05$), the free triidothyronine and thyroid hormone levels of the patients ≤40 years old were significantly higher than those of those older than 40 ($P<0.05$), the triidothyronine levels of the patients complicated with diabetes were significantly lower than those not ($P<0.05$), and the free triidothyronine levels of the blood lipid metabolic disorder patients were significantly than the normal ones ($P<0.05$). After excluding the influences of gender, age and diabetes, the levels of triidothyronine were positively correlated with those of total cholesterol and LDL-C, and the levels of free triidothyronine were positively correlated with those of LDL-C.

Conclusion: The thyroid hormone levels of mental disorder patients were elevated in case of blood lipid metabolic disorders.

Key words: Mental disorder, thyroid hormone, hyperlipemia.

Introduction

With the rapid development of modern society and accelerated pace of life, people are suffering from increasing stress. The incidence of mental disorder is significantly higher than ever before. Currently, a large number of studies have confirmed that psychiatric patients may experience significantly increased glucose and lipid metabolic abnormalities, weight gain and metabolic syndrome and larger cardiovascular morbidity and mortality risk in the antipsychotic treatment compared with those previously [1-3]. Thyroid hormones, which include two active types triiodothyronine (T3) and thyroxine (T4), are important endocrine hormones that can regulate body growth and metabolism. The metabolic disorders of thyroid hormones may induce a series of changes in blood lipid, body weight, blood pressure and blood sugar. At present, the serum thyroxine level and blood lipid metabolism in psychiatric patients have barely been studied [4-6]. This study aims to explore the relationship between the serum thyroid hormone levels and blood lipid in psychiatric patients.

Materials and methods

Subjects

The psychiatric patients who were diagnosed and treated in our hospital from June 1 to December 31 in 2011 were selected. The inclusion criteria were as follows: ① the diagnosis of all patients was in line with the third edition of Classification and Diagnostic Criteria of Mental Disorders in China (CCMD-3). The contents of the diagnosis mainly included schizophrenia and related disorders, organic mental disorders, mental disorders induced by psychoactive substances, emotional disorders and mental development retardation, etc.; ② all patients were hospitalized for no less than 6 weeks. Exclusion criteria: the patients who took thyroid hormone drugs for thyroid disease or who have recently been given anti-thyroid medication were excluded.

Methods

The self-designed questionnaire was adopted for the collection of basic clinical data of patients, including gender, age, hospital stay this time, previous history of physical disease, history of drug use (antihypertensive drugs, hypoglycemic drugs, lipid-lowering drugs), and monitor the patients on the levels of fasting blood sugar, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), triglyceride (TG), fasting serum triiodothyronine (TT3), thyroxine (TT4), thyroid stimulating hormone (TSH), free triiodothyronine (FT3) and free thyroxine (FT4). According to the lipid levels, the patients were divided into hypertriglyceridemia (TG≥ 26 mmol/L), hypercholesterolemia (TC ≥ 22 mmol/L), low- and high-density lipoprotein hyperlipidemia (HDL-C < 1. 04 mmol/L) in accordance with the China Adult Dyslipidemia Prevention Guide (2007 Edition) [7].

Statistical analysis

All data were analyzed by SPSS 17.0. The numeration and measurement data were subjected to parameter- and non-parameter- analyses, including t test, χ^2 test, correlation analysis and etc. P<0.05 was considered statistically significant.

Results

General clinical data

The detailed clinical data of patients are shown in Table 1. The patients included 133 males and 83 females, aged between 14 and 67 years old, with the average age of (51.36 ± 11.37) years old. Their hospital stays ranged from 6 to 167 days, with an average of (73 ± 9.14) d. The specific subtypes of the diagnosis of psychiatric disorder were as follows: schizophrenia and its related disorders in 180 cases, mental disorders induced by psychoactive substances in 14 cases, organic mental disorders in 11 cases, affective disorders in 5 cases and mental development retardation and other disorders in 6 cases. The methods of antipsychotic treatment were as follows: 77 patients were given combined therapy with two kinds of antipsychotics, and 149 with an antipsychotic drug, of which 22 patients received chlorpromazine and other classic antipsychotic treatments and 91 patients were administered with clozapine, risperidone and etc. The average therapeutic dose of antipsychotics (chlorpromazine effective dose equivalent [8-10]) was (306. 04 ± 201. 32) mg/d. 28 and 61 psychiatric patients were complicated with diabetes and dyslipidemia respectively, in which the lipid levels of 7 patients was recovered after lipid lowering therapy, and the remaining 54 cases were classified simply based on the clinical test results: 10 cases of hypertriglyceridemia (4.6 %), 6 cases of hypercholesterolemia (2.7%) and 38 cases of low- and high-density lipoprotein hypercholesterolemia (17.6%). The average level of fasting serum TT3 was (0.78 ± 0.24) ng/ml, TT4 (7.51 ± 1.66) μg/ml, TSH (2.57 ± 1.68) μIU/ml, FT3 (2.02 ± 0.47) pg/ml and FT4 (1.24 ± 0.26) ng/ml.

Table 1 shows that the serum TT3 level of male patients was significantly higher than that of females (P <0.05), and the levels of serum FT3 and FT4 of patients 40 years old or younger was significantly higher than those of the patients above 40 (P <0.05). Meanwhile, the level of serum TT3 of the patients accompanied by type 2 diabetes was lower than those without diabetes, and the serum FT3 level of the patients accompanied by abnormal lipid metabolism was higher than those without abnormalities, between which the difference was statistically significant (P <0.05).

Relationship between antipsychotic and thyroid hormone level

The specific conditions of antipsychotic drugs and thyroid hormone levels are summarized in Table 1. We found that there were no statistically significant differences in the thyroid hormone levels of the patients who received single 1st generation and 2nd generation antipsychotics, as well as the combined medication (P> 0.05).

Correlation analysis of thyroid hormone level and blood lipid metabolism

The correlation analyses of thyroid hormone levels and lipid metabolism are listed in Table 2. We found that after excluding the influencing factors such as gender, age and diabetes, the serum TT3 level was positively correlated with the TC and LDL-C levels (P <0.05), and the serum FT3 and LDL-C levels were positively correlated (P <0.05).

Table 1. Serum thyroid hormone levels of the patients (x±s)

Item	Gender		t value	P value
	Male (n=133)	Female (n=83)		
TT (ng/ml)	0.81±0.25	0.72±0.21	2.841	0.006
FT (pg/ml)	2.13±0.45	1.98±0.45	1.195	0.236
TT (ug/ml)	7.39±1.41	7.71±1.75	-1.197	0.231
FT (ng/ml)	1.03±0.27	1.09±0.25	-1.021	0.314
TSH (uIU/ml)	2.39±1.61	2.85±1.79	-1.797	0.091
Item	**Age**		**t value**	**P value**
	≥40 (n=163)	<40 (n=53)		
TT (ng/ml)	0.75±0.23	0.84±0.22	-1.881	0.066
FT (pg/ml)	1.99±0.42	2.23±0.57	-2.072	0.014
TT (ug/ml)	7.61±1.55	7.21±1.72	1.411	0.165
FT (ng/ml)	1.04±0.25	1.15±0.25	-2.556	0.014
TSH (uIU/ml)	2.62±1.61	2.61±1.73	0.151	0.889
Item	**Blood lipid**		**t value**	**P value**
	Normal (n=145)	Abnormal (n=71)		
TT (ng/ml)	0.75±0.23	0.79±0.21	-1.031	0.311
FT (pg/ml)	1.99±0.41	2.15±0.51	-2.057	0.052
TT (ug/ml)	7.55±1.72	7.44±1.25	0.437	0.668
FT (ng/ml)	1.05±0.24	1.19±0.23	-0.716	0.479
TSH (uIU/ml)	2.64±1.83	2.44±1.41	0.822	0.411
Item	**Type 2 diabetes**		**t value**	**P value**
	No (n=161)	Yes (n=55)		
TT3 (ng/ml)	0.75±0.23	0.71±0.61	-0.249	0.051
FT3 (pg/ml)	2.02±0.46	1.95±0.41	-1.067	0.292
TT4 (ug/ml)	7.49±1.42	7.69±1.23	0.706	0.478
FT4 (ng/ml)	1.07±0.26	1.07±0.23	0.216	0.819
TSH (uIU/ml)	2.60±1.63	2.44±1.87	-0.462	0.651

Item	Drug treatment			F value	P value
	Single 1st generation antipsychotic (n=126)	Single 2nd generation antipsychotic (n=56)	Combined antipsychotics (n=34)		
TT3 (ng/ml)	0.75±0.23	0.75±0.23	0.78±0.26	1.861	0.161
FT3 (pg/ml)	1.96±0.43	2.02±0.47	2.11±0.43	1.269	0.285
TT4 (ug/ml)	7.46±1.57	7.66±1.59	7.39±1.43	2.216	0.114
FT4 (ng/ml)	1.04±0.16	1.07±0.26	1.04±0.23	0.436	0.656
TSH (uIU/ml)	2.63±0.97	2.53±1.55	2.44±2.57	0.086	0.921

Table 2. Correlation analysis of thyroid hormone level and blood lipid metabolism

Item		TT3	FT3	TT4	FT4	TSH
TC	r	0.206	0.372	-0.015	-0.036	-0.096
	P	0.009	0.632	0.886	0.626	0.243
TG	r	0.097	0.294	0.146	0.035	-0.067
	P	0.257	0.711	0.069	0.669	0.389
LDL-C	r	0.170	0.159	0.009	0.007	-0.041
	P	0.029	0.049	0.875	0.956	0.581
HDL-C	r	-0.011	-0.013	-0.146	-0.154	0.093
	P	0.211	0.242	0.058	0.059	0.239

Discussion

Influences of age and gender on thyroid hormone level

There have been a large number of epidemiological information showing that age can reduce the serum TT3 and FT3 levels in psychiatric patients. Chen et al. [11-13] observed that the serum FT4 level of males is slightly higher than that of females. Franco et al. [14, 15] found that age is an independent factor affecting the serum FT3 level in patients with type 2 diabetes. This study found that the serum TT3 level of male patients was significantly higher than that of female patients ($P < 0.05$), and the serum FT3 and FT4 levels of patients at or less than 40 years old were significantly increased compared with those above 40 years old ($P < 0.05$), which indirectly confirmed the above results, indicating that age and gender are key factors influencing thyroid hormone in psychiatric patients.

Influences of antipsychotic on thyroid hormone level

Researchers believe that antipsychotics can block dopamine (DA) and 5 - hydroxyl tryptamine (HT) receptors to inhibit pituitary-thyroid axis, so as to cause changes in serum thyroid hormone levels. Relevant studies have not reached a consensus yet. Pesek et al. [16, 17] studied psychiatric patients who were treated with chlorpromazine, risperidone and quetiapine for 4 weeks, and found that chlorpromazine can cause a decline in the levels of thyroid hormones, quetiapine can elevate thyroid hormone levels, and risperidone has no effect on thyroxine. Kontaxakis et al.[18] has confirmed that the increase of thyroid hormone levels caused by the use of quetiapine can be recovered to normal 6 weeks after treatment interruption. Scorza et al. [19] holds that the high incidence of cardiovascular diseases in schizophrenia patients is related to abnormal glucose and lipid metabolism and subclinical hyperthyroidism. This study did not find that there was statistically significant difference in the serum thyroid hormone levels of psychiatric patients between single medication and combined medication; according to analysis, it is believed that it is possibly due to a small sample size, the effects of each antipsychotic drug on thyroxine level cannot be explored singly.

Influences of type 2 diabetes on thyroid hormone level

There have been literatures [20, 21] confirming that thyroid hormones play an important role in metabolism in the body. Small-dose thyroid hormones may promote glycogen synthesis, while high-dose thyroid hormones can inhibit glycogen synthesis, and promote the degradation of glycogen, therefore, relatively high levels of serum thyroid hormones in vivo can aggravate diabetes; in turn, abnormal glucose metabolism may also affect the levels of thyroid hormones, hyperglycemia associated with decreased levels of thyroid hormones. The study on psychiatric patients associated with type 2 diabetes found that poor glycemic control may result in decreased serum FT3; fasting glucose is an independent factor of FT3 change. The serum TT3 of psychiatric patients with type 2 diabetes was lower than that of patients without diabetes ($P < 0\ 05$), consistent with the results of the study on diabetic patients.

Influences of blood lipid metabolism on thyroid hormone level

Thyroid hormones can promote lipid synthesis and degradation, and the latter particularly reduces blood lipid levels. The clinical study found that before treatment, the blood lipid level of patients with hyperthyroidism was lower than that of the patients in the control group, but the level of patients with hypothyroidism was higher than that of the patients in the control group. Zhang et al. [22] studied the relationship between the lipid level and thyroid hormones of persons with normal thyroid function, and found that FT4 rose with the increase of TC. The results of this study are similar, which are mainly manifested in the increased levels of TT3 and FT3, indicating that the thyroid hormone levels of psychiatric patients rose correspondingly, accompanied by lipid metabolic abnormalities. Bryzgalova et al. [23, 24] further confirmed that KB-141 can not only reduce 6% to 8% of the body weight, but also reduce blood lipid levels, improve glucose tolerance abnormality, which is possible to prevent the occurrence of type 2 diabetes. Grover et al. found that the selective agonist of thyroid hormone receptor subtypes (TRbeta) KB-141 can increase the primate basal metabolic rate and lose weight, without the side effect of tachycardia. At present,

a large number of animal studies have confirmed that thyroid hormones can reduce blood lipid levels mainly through inhibiting cholesterol absorption and increasing basal metabolism and many other ways[25-27]. However, the influence of dyslipidemia on serum thyroid hormone levels and its mechanism have been rarely reported, so it is required to increase sample size combined with animal experiments for further studies in the future.

The inadequacies of this study lie in the relatively small sample size selected and relatively long antipsychotic treatment, which only reflects the correlation between abnormal lipid metabolism and thyroid hormone levels. It is pending to conduct prospective studies with large sample size to further explore the changes of thyroid hormones in psychiatric patients.

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Results of the effect of octenidine dihydrochloride 0.1% and phenoxyethanol 2% on rat CNS in the experimental cranial surgery

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Abstract

Purpose: Cranial and spinal emergency procedures are performed to treat a number of neurosurgical disorders. Infection is one of the most serious complications after several emergency procedures. The purpose of this study was to investigate the morphological and neurotoxic effects of Octenidine Dihydrochloride 0.1% and Phenoxyethanol 2% (OCT), which is one of the antiseptic agents.

Methods: Forty rats were randomly divided into four groups and underwent craniectomy under surgical microscope: sham group (n=10); control group (n=10) received normal saline solution; dura mater-intact study group (n=10) put drop OCT locally (epidural space) in 100% concentration; menings-dissected study group (n=10) put drop OCT locally (submeningeal space) in 100% concentration. After scarification on 30th day, each sample was evaluated for regular meningeal continuity, vascular proliferation, polymorph nuclear leukocyte infiltration, foreign body reaction, neural degeneration, and cortical cerebral ischemia.

Results: In the presented study, no adhesions or fibroses were observed between the scalp and dura mater at the craniectomy site. The dura maters were intact in Groups 1, 2, and 3. The menings did not have any continuity in Group 4. All the specimens did not reveal any neural degeneration and cortical ischemia. In addition, no inflammatory cell infiltrations were observed and no foreign body reactions or granulomas were noted. HE and CD31 antibody staining did not show any vascular proliferations.

Conclusion: OCT may be safely used in neurosurgical procedures especially gunshot injury and open depressed skull fracture. Further investigations will be necessary to determine Octenisept's probable dose-dependent effectiveness and long term complications.

Key words: Antiseptic, Emergency, Neurotoxic, Octenidine, Phenoxyethanol, Surgery.

Introduction

In neurosurgical practice, several different procedures are performed to treat a number of emergency cranial and spinal surgeries such as trauma, gunshot injuries of open field battle. Surely, some infection based complications still arise in 3–9% of neurosurgical ICU patients and 0.4–2% of all patients hospitalized with head trauma [1-3]. In infection management, local and/or systemic application of antibiotics and removal of the foreign bodies, cranioplasty kits and evacuation of abscesses are the main stay.

On the other hand, local antiseptic agents are effective in management of treatment procedures [4,5]. However, applications of these agents are problematic as their neurotoxic effects are not clear. This study, therefore, aimed to investigate the neurotoxicity of 0.1% octenidine dihydrochloride (OD), 2% 2-phenoxyethanol (2-PE) - an option of during surgery application for infection control of open cranial wound- which also preferred by general surgeons, plastic surgeons, etc. in the management of such type of bactericidal and fungicidal infections.

Material and Methods

In this study, 40 randomized female Sprague – Dawley rats weighing 250-350g were studied. All the animal experimental procedures were approved by Gulhane Military Medical Academy Animal Care and Use Committee (22/05/2009-09/41K) and were carried out in accordance with the guidelines of the National Institute of Health on animal care and related ethics. The study was planned according to STAIR guidelines [6]. Ketamin hydrochloride (Ketalar, Parke-Davis, England), 100 mg/ kg

and xylazine 5 mg/ kg were given to the rats intramuscularly. Skins of all rats were cleaned with 1puff dosage of Octenidine Dihydrochloride 0.1% and Phenoxyethanol 2% (OCT) (1,5ml/100cm^2). The rats were randomly divided into four groups and underwent craniectomy (0,25cm^2) under surgical microscope. The lethal dosage (LD_{50}) following oral administration is 1,3 to 3,4 g/kg body weight (b.w) (rat); following dermal application is 13 ml/kg b.w (rat); following single intravenous administration is 10 mg/kg b.w and following intraperitoneal administration is 10-12 ml/kg [7]. Groups were listed as Sham group (n=10); control group (n=10) put drop normal saline solution; duramater-intact study group (n=10) put drop 9 ml/kg b.w OCT, locally (epidural space) in 100% concentration; menings-dissected study group (n=10) put drop 9 ml/kg b.w OCT, locally (submeningeal space) in 100% concentration. The rats were fasted overnight with free access to water. The rats were given anesthesia with Ketalar 100 mg/ kg and xylazine 5 mg/kg intramuscular and sacrificed by decapitation on the 30th day after craniectomy. The brains and the dura maters were removed from the skull. Each specimen was stored in 10% neutral buffered formaldehyde solution for 24 hours at room temperature. Each sample was embedded in a paraffin block by a Standard method. Sections of 3-µm thickness were cut and stained with Haematoxylin and eosin (HE) for light microscope evaluation. Each sample was evaluated for neurodegeneration with silver stain. The samples were studied blindly by a pathologist for the evaluation of the dura mater and cerebral cortex. Each sample was evaluated for regular meningeal continuity, vascular proliferation, polymorph nuclear leukocyte (PNL) infiltration, foreign body reaction, neural degeneration, and cortical cerebral ischemia. Detailed immunohistologic investigation of inflammatory cell infiltration and vascular proliferation were observed by CD31 (Cell Marque Corporation-Rocklin, CA,USA, dilution factor 1:25), CD68 (Cell Marque Corporation-Rocklin, CA,USA, dilution factor 1:100), LCA (Cell Marque Corporation-Rocklin, CA,USA, dilution factor 1:100), and CD15 (Cell Marque Corporation-Rocklin, CA,USA, dilution factor 1:25). The immunohistochemical staining was performed automatically with Ventanas Benchmark® XT.

Results

Each rat was observed individually in a separate cage, and no neurobehavioral deterioration was observed in any of the rats throughout 30 days. After the sacrifice of rats, no adhesions or fibrosis were observed between the scalp and dura mater at the craniectomy site. In addition, no perforations in the dura mater (except in Group 4 where the menings was dissected according to the study) were noted macroscopically.

In the evaluation with a light microscope, the menings of the rats were intact (n=30) except in Group 4 (n=10). The dura mater was not thickened in Groups 1, 2, and 3 (n=30). All the specimens from Groups 1, 2, and 3 were evaluated with both HE and immunohistologic staining and did not show any neural degeneration and cortical ischemia. Moreover, no inflammatory cell infiltrations, foreign body reactions, or granulomas were observed (Figure 1).

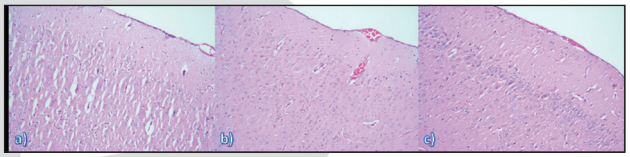

Figure 1. Sections of cerebral cortex and dura mater. Haematoxylin and eosin (HE) staining (x100) shows the continuity of dura mater. a) Group 1, b) Group 2, c) Group 3.

Iatrogenic interruption of meningothelial lining has been shown in Group 4 and also there was no cortical ischemia (Figure 2).

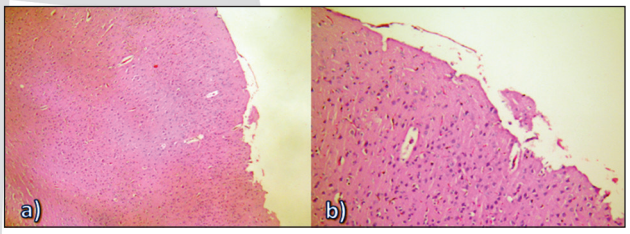

Figure 2. In Group 4, iatrogenic interruption of meningothelial lining has been shown in this figure. And also, there is no cortical ischemia. a) HE, x40 b) HE, x100

When silver-stained samples were evaluated, the comparison with Group 3-4, and Group 1-2 has not been determined any neurodegeneration (Figure 3).

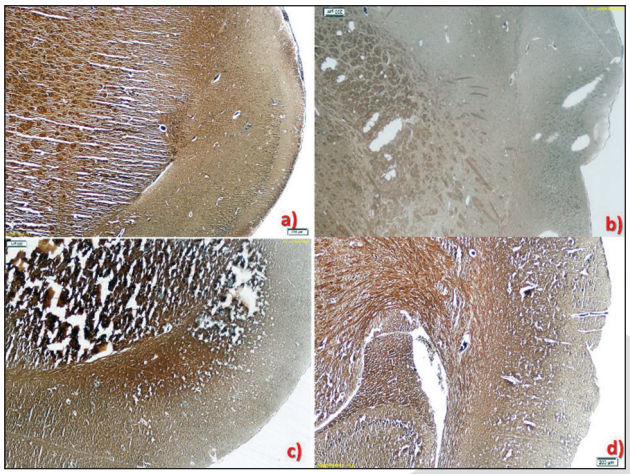

Figure 3. Silver staining. In the section of each group, there is no neural degeneration. (Methenamine silver, x40) a) Group 1, b) Group 2, c) Group 3, d) Group 4

In addition, no inflammatory cell infiltrations were observed at the HE and immunohistologically (LCA, CD15, CD68 antibody) (Figure 4) stained sites, and no foreign body reactions or granulomas were noted. HE and CD31 antibody staining (Figure 5) did not show any vascular proliferations.

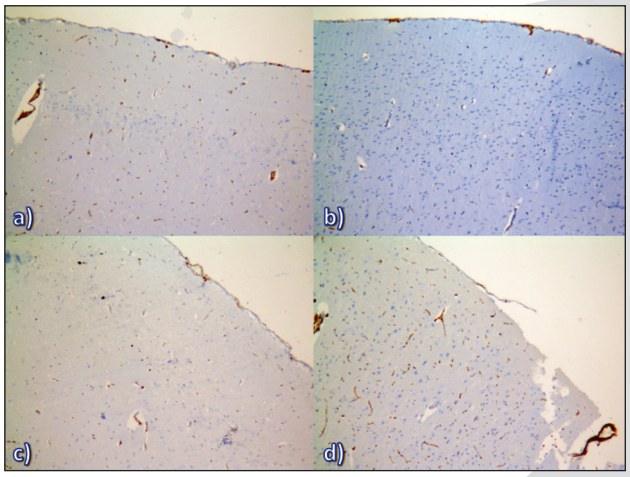

Figure 4. Immunocytochemical findings of dura mater and cerebral cortex. In the sections, there are not any significant PMNL infiltrations. (LCA, x100) a) Group 1, b) Group 2, c) Group 3, d) Group 4

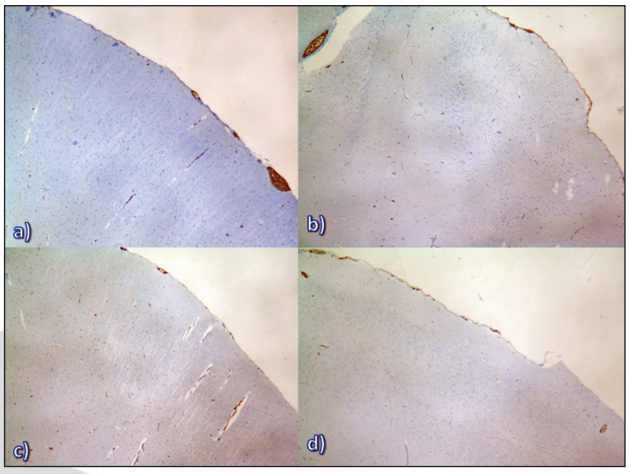

Figure 5. CD31 antibody staining shows no vascular proliferation in the Groups. (x40) a) Group 1, b) Group 2, c) Group 3, d) Group 4

Discussion

Although decompressive craniectomy is a potentially lifesaving procedure used in the treatment of trauma, traumatic brain injury, gunshot wound, intracerebral hemorrhage, malignant edema, etc., but it has some disadvantages. The patients need to undergo a second procedure for cranial defect repair [1]. The procedure is conducted with a few variations such as the timing of surgical intervention, techniques, materials and kits, and storing the bone flap prior to reconstruction [8]. Some complications of emergency surgeries and cranioplasty are wound and postoperative infections (with foreign body implantation), subdural or epidural fluid accumulations, seizures, and/or fixed neurological deficits [2,8,9]. The occurrence of the complications is bothersome for both the physician and the patient.

The patients with complications such as infection require another operation to address their previous surgical procedure. Especially, infection after cranioplasty necessitates removal of the cranioplasty kit [9,10]. Multiple antibiotic applications with hyperbaric oxygen therapy are the second step of the infection treatments indicating the importance of prophylaxis in neurosurgical practice [1]. Although prophylactic antibiotics are in use in neurosurgical practice, no data are available on the use of antiseptic agents. Previous studies with OCT have shown it to be effective in inhibiting the growth of plaque forming bacteria and in reducing development of plaque in both experimental animals and humans [11].

OCT was originally developed as a potential broad-spectrum topical antimicrobial (bactericidal and fungicidal) and antiseptic agent, which is an active biocide at low concentrations (0.1% and below). OCT is an effective antimicrobial on various micro-organisms [12-15]. It is particularly effective on Staphylococcus aureus, meticillin-resistant Staphylococcus aureus (MRSA), Staphylococcus epidermidis, Klebsiella pneumoniae, Serratia marcescens, Streptococcus pyogenes, Serratia marcescens, and Pseudomonas aeruginosa colonization [11,16,17]. OCT solution represents an effective topical therapy of Gram negative infections [11,17].

Zumtobel et al. suggested that OCT coating of tracheotomy tubes showed an antimicrobial effect and reduced colonization and biofilm formation on polymer tracheotomy tube surfaces [15]. The chemical, antimicrobial, and toxicological properties of OCT have been described previously. In addition, earlier studies demonstrated its activity against dental plaque micro-flora and on skin micro-flora; our findings demonstrated that OCT (an aqueous solution containing 0.1% OD and 2% 2-PE) did not have any neurotoxic effects on CNS and could be used safely on the dura mater [11,18,19].

There are many questions concerning the influence of antiseptics on neurotoxicity due to exposure to free radical mediated oxidation. Although antiseptic agents are used to reduce the incidence of infections, these agents also affect the healthy tissues and blood cells. OCT is a potential non-alcoholic mucous skin and wound antiseptic, which destroys bacterial cells by interacting with their cellular wall and intracellular components [7]. Although Hupuczi et al and Parlakgumus et al. have suggested that OCT solution used for peritoneal irrigation plays a role in tissue toxicity that causes chemical serositis with effusion [20,21]. In our experimental study; we did not observe any clinical side effect such as adhesion, fibrosis, and cerebritis or determine any histopathological changes in the neural tissue when OCT was applied on either the dura mater or the cerebral cortex.

OCT is still in usage as an effective antiseptic in general surgery, plastic surgery and gynecologic procedures. Both antiseptic and anti-adhesive effects on peritonitis are advantages of the solution [13,15,22]. Despite potential benefits of OCT in neurosurgical practice as an antiseptic, its neurotoxic effects should be evaluated primarily and it should also be shown that it has no morphologic effects on the dura mater and/or the cerebral tissue. Literature presents limited information on neurosurgical practice with OCT. Thus, the presented study aimed to evaluate the efficiency and reliability of OCT in Neurosurgery.

Conclusion

This is the first study about OCT in Neurosurgery. OCT may be safely used in neurosurgical emergency procedures (gunshot injury, open depressed skull fracture, spinal trauma, etc.). Further investigations will be necessary to determine probable dose-dependent effectiveness and long term complications.

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Effects of delayed surgery on mortality and morbidity in geriatric patients with hip fracture

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Abstract

Background and aim: Hip fractures are associated with a high rate of mortality and profound temporary and sometimes permanent impairment of quality of life. The effects of delaying hip fracture surgery on mortality and morbidity in elderly patients are not completely understood. This study therefore aims to identify the rate of mortality in geriatric patients underwent surgical treatment of the hip fractures in relation to the general population of the same age group in our environment and to correlate the timing of operative intervention with the mortality and morbidity rate.

Patients and methods: In this a retrospective-prospective study we studied two groups of patients older than 65 years who were operatively managed for a hip fracture. Patients in the first group were operated within 48 hours from hospital admission, and patients in the second group had delay in receiving surgical treatment of more than 48 hours. We extracted patient age, sex, ASA score, fracture classification, treatment modality, types of anesthesia, perioperative blood transfusion, complications and cause of death; followup was 6 months.

Results: Overall mortality during the first 6 months after hip fracture surgery was 13,3% with cardiovascular diseases as the main cause of death. Mortality was significantly more frequent in patients who had surgical delay of more than 48 hours (20%) compared with patients who had surgery within 48 hours from admission to hospital (6,7%). Patients with ASA 3 and ASA 4 scores and male sex had higher mortality rate after hip fracture. We found also a larger number of complications in the group with a delay in surgical treatment.

Consclusion: The risk of mortality in hip fracture patients was almost three fold higher than that in relation to the general population of the same age group. Patients who underwent surgery within 48 hours after the injuries have a significantly lower rate of mortality and morbidity compared to patients whose surgery was postponed for more than 48 hours after the injury.

Key words: hip fracture, delayed surgery, geriatrics, mortality, morbidity

1. Introduction

A steady increase in the number of patients with hip fractures is registered in the world. According to estimates, this number could reach 2.6 million hip fractures per year until 2025 and 25.6 million by 2050, mostly due to prolonged life expectancy and an increase in the proportion of elderly population in most countries, but it also suggests a reversal of this trend due to the more effective prevention measured (1).

Hip fracture is associated with significant morbidity, loss of independence and reduced quality of life (2). The general rate of mortality associated with hip fracture is higher than the better known life-threatening pathological conditions such as gastric or pancreatic cancer or myocardial infarction (3,4). Given the high rate of six months (11-23%) and one-year mortality (22-29%) (5) and well-known high incidence of hip fractures in the geriatric population, hip fractures may contribute to the relatively large proportion of deaths in the general population. As the age, malnutrition and general health status represents precisely identified risk factors (6,7), research results on influence of the patient's sex and the interval between injury and surgery on the mortality of geriatric patients with hip fractures remain controversial.

According to current guidelines surgery for patients with hip fractures should be performed within 24 hours after admission because it is believed that the earlier operative treatment compared to delayed increases the survival rate (8). Still lack sufficient

evidence to clearly define the algorithm by which should be determined the minimum period required for preparation for surgery after the injury because of ethical reasons the influence of delaying surgery on morbidity and mortality cannot be determined via randomized, controlled studies.

2. AIMS

We analyzed the rate of mortality and morbidity in geriatric patients with hip fractures in patients who underwent surgery within 48h after injury compared to patients operated after this period, and the total mortality rate compared to the general population of the same age group.

3. Patients and methods

This study was clinical, observational, cross-section, retrospective-prospective and was performed at the Clinic of Orthopedics and Traumatology, Clinical Center of Sarajevo University in the period from January 1st 2010 to December 31st 2011.

After obtaining ethical approval the study included male and female patients aged over 65 years, which underwent surgical treatment of hip fractures (with additional inclusion/exclusion criteria). Respondents were screened by random sampling, while the sample for testing implied choice of 60 subjects, who were by cross-sectional method, divided into two groups according to the time until surgical treatment. Time to surgical intervention (hours) was calculated as the difference between the date of hospitalization and the date of the surgery, as follows: Group A - 30 patients aged over 65 years in which surgery for fixation of hip fracture was made within 48 hours after hospital admission and Group B - 30 patients aged over 65 years in which surgery of hip fracture fixation was performed after 48 hours after hospital admission

All subjects were followed by the same protocol, with the prophylactic use of low molecular weight heparin from hospital admission and prophylactic administration of antibiotics at the day of surgery. Type of anesthesia is determined by the decision of the responsible anesthesiologist, in consultation with the patient, taking into account the indications and contraindications for certain types of anesthesia and the type of surgical procedure is determined by the type of fracture and operator preferences. Postoperatively, a rapid intensive physiotherapy was initially applied to all patients.

Primary outcomes of the study were mortality, which is defined as any cause of death within 180 days after the surgery, compared to the general population of the same age group, the causes of mortality and the number of postoperative complications with analysis of correlation between interval injury-surgery, gender and ASA *(American Society of Anesthesiologists score)* with the mortality. Postoperative complications were verified and documented by clinical examination, diagnostic examinations and laboratory tests by the orthopedist/anesthesiologists or other specialists during medical consultations.

4. Results

Analysis of the patients characteristics (Table 1) shows that there were similar between the two groups ($p>0.05$), confirming the homogeneity of the surveyed groups, except for the analysis period between admission and the surgery. Specifically, with regard to the formation of the groups is observed a statistically significant difference ($p<0.05$) in the period from admission to the surgical treatment of hip fractures: Patients in Group A on average spent 36.4±13.9 hours from admission to surgery, while the patients from Group B spent 162.4±190.9 hours. The average age in Group A was 75.03±5.8 years, while in Group B it was 76.97±7.7 years, while in both groups were overrepresented women - in Group A 76.7% and in Group B 73.3%. In both groups were more present intracapsular fractures, in Group A in 56.7% and in Group B in 53.3% of cases, compared to extracapsular which were represented by 43.3% and 46.7. Most of the respondents had an ASA 3, in Group A - 53.3% and in Group B - 56.7%. In Group B was recorded higher percentage of ASA 4 - 20%, compared to Group A - 13.3%. In Group A, a higher percentage of respondents had ASA 2 - 33.3%, compared to Group B in which 23.3% of patients had ASA 2. In both groups, the general anesthesia was dominant or in Group A with a share of 86.7% and 93.3% in Group B, while spinal anesthesia was applied in 13.3% of cases in Group A and 6.7% in Group B. In both groups PEP (partial endoproth-

esis) was most frequent or in group A with 56.7%, while in group B with 46.7%. DHS (dynamic hip screw) and TSHS/Sa (two screw holl system) in both groups were equally represented in percentage with 36.7% (DHS) and 6.7% (TSHS/Sa). Gamma nail as the surgical technique has been applied only in Group B with a share of 10%.

The highest percentage of respondents had received two doses of RBC (red blood cell), in Group A - 40% and in Group B - 33.3%. In Group A or one dose RBC did not receive 36.7%, while in Group B - 20% of respondents. Three and four doses of RBC received 6.7% patients in group A, while in group B, three doses of RBC received 16.7%, and four doses RBC 10%. In contrast to group A, in which none of the respondents had received five or six doses of RBC, in Group B this percentage is 6.6%.

During the six-month follow-up a total of 13.3% of patients had lethal outcome, with more cases reported in the Group B (20%) compared to Group A (6.7%) ($p<0.05$) (Table2). The most prevalent postoperative complications in both groups were: respiratory diseases (16.6%), circulatory system disease (10.0%), urinary tract infection (8.3%) and acute confusional state (6.7), which were more frequent in Group B ($p<0.05$) (Table 3). The most common cause of death in both groups were circulatory system disease (6.7%), then with equal representation gastrointestinal diseases (1.7%), malignancies (1.7%) and one unknown cause of dead (1,7%) without difference between the groups ($p>0.05$) (Table3).

In Group A there were no gender differences among patients with lethal outcome, as opposed to group B where 13.3% of men had died compared to 6.7% of deaths among female patients (Figure 1) ($p <0, 05$). In the baseline sample is observed a higher prevalence of ASA score 3 and 4 in patients with

Table 1. Patient characteristics

		Group A	Group B	p-value
		N (%)	N (%)	
Age in years, mean (SD)		75,03 (5,8)	76.97 (7,7)	0,275
Gender	Male Female	7 (23,3%) 23 (76,7%)	8 (26,7%) 22 (73,3%)	0,500
Types of fracture	Extracapsular Intracapsular	13 (43,3%) 17 (56,7%)	14 (46,7%) 16 (53,3%)	0,500
Time to surgery in hours; mean (SD)		36,4 (13,9)	162,4 (190,9)	0,001
ASA score	2 3 4	10 (33,3%) 16 (53,3%) 4 (13,3%)	7 (23,3%) 17 (56,7%) 6 (20,0%)	0,619
Types of anesthesia	General Spinal	26 (86,7%) 4 (13,3%)	28 (93,3%) 2 (6,7%)	0,335
Types of surgery	DHS Gamma nail PEP TSHS/Sa	11 (36,7%) 0 (0) 17 (56,7%) 2 (6,7%)	11 (36,7%) 3 (10,0) 14 (46,7%) 2 (6,7%)	0,349
RBC transfusion (No of doses)	0 1 2 3 4 5 6	11 (36,7%) 3 (10,0%) 12 (40,0%) 2 (6,7%) 2 (6,7%) 0 (0%) 0 (0%)	6 (20,0%) 4 (13,3%) 10 (33,3%) 5 (16,7%) 3 (10,0%) 1 (3,3%) 1 (3,3%)	0,508

SD = standard deviation; ASA score= American Society of Anesthesiologists score;
DHS = dynamic hip screw; PEP = partial endoprothesis; TSHS/Sa = two screw holl system;
RBC= red blood cell

Table 2. Six-month postoperative mortality

Group A Group B Total					
Died	Yes	N	2	6	8
		%	6,7	20,0	13,3
	No	N	28	24	52
		%	93,3	80,0	86,7
Total		N	30	30	60
		%	50,0	50,0	100,0

p<0.05

Table 3. The most prevalent postoperative complications and underlying cause of death

	Group A	Group B	Total
	N (%)	N (%)	N (%)
Postoperative complications*			
Respiratory disease	4 (3,3)	8 (26,7)	10 (16,6)
Circulatory system disease	2 (6,7)	4 (13,3)	6 (10,0)
Urinary tract infection	1 (3,3)	4 (13,3)	5 (8,3)
Acute confusional state	1 (3,3)	3 (10,0)	4 (6,7)
Deep infection	0 (0)	2 (6,7%)	2 (3,3)
Underlying cause of death**			
Circulatory system disease	1 (3,3)	3 (10,0)	4 (6,7)
Respiratory disease	0 (0)	1 (3,3)	1 (1,7)
Gastrointestinal disease	1 (3,3)	0 (0)	1 (1,7)
Malignancy	0 (0)	1 (3,3)	1 (1,7)
Unknown	0 (0)	1 (3,3)	1 (1,7)

* *p<0.05*
** *p>0.05*

lethal outcome (62.5% and 37.5%), compared to those who survived among whom there was a higher percentage of ASA 2 (32.7%) (p<0.05) (Figure 2).

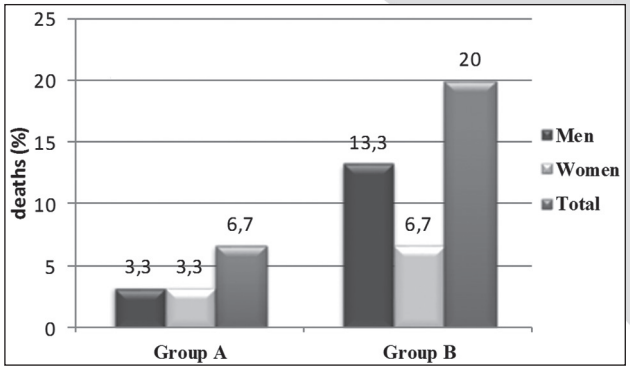

Figure 1. Sex differences in postoperative mortality

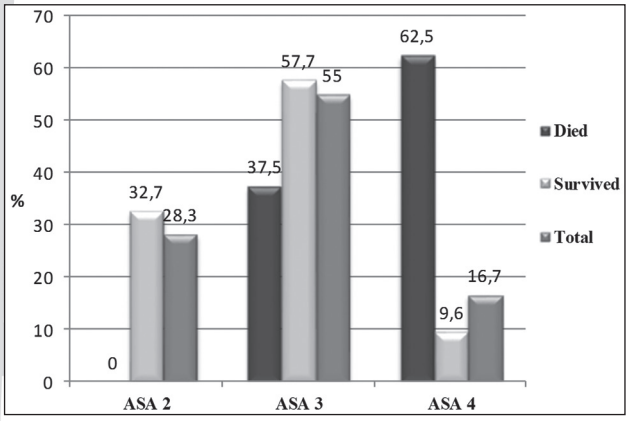

Figure 2. ASA score according to total mortality

5. Discussion

According to data from the The Federal Office of Statistics of Federation of Bosnia and Herzegovina for 2010, mortality among those aged over 65 years amounted to 4.6%, and by comparison with our results, we see that the percentage of the total mortality of geriatric patients after hip

fracture surgery is almost three fold higher. Same results of increased mortality during the study period compared to the general population have shown study by Panula et al. (2011) and De Luise et al. (2008), reporting that the risk of mortality in hip fracture patients during the study period was 3-fold higher than that in the general population (9,10). Also systematic epidemiological review by Abrahamsen et al. (2009) (11), reports that 12 studies demonstrated an increased mortality rate compared to population-controlled cohort group, including the first year after the occurrence of fractures. By the Australian population-based study, the main cause of death after hip fracture were diseases of circulatory system in approximately 45% of cases, respiratory diseases in 10.8%, and neoplasms in 10.7% of cases. According to the death certificates, mortality due to hip fractures may be underestimated because of hip fractures as a cause of death are mentioned in less than 2% of all deaths and as a contributing cause of death in 21% of cases (12).

In the available literature there is still ongoing debate about the acceptable definitions which period of time from the admission is an „unacceptable delay" of the surgery. Current guidelines (8) recommend that patients with hip fractures should be subjected to surgical treatment within 24 hours after the injury, because it is assumed that the earlier operative treatment is associated with better functional recovery, shorter hospitalization, shorter duration of pain and a lower rate of postoperative complications and mortality (13,14). On the other hand, delaying the surgery may allow better optimization of the patient's health status, and thus lower the risk of perioperative complications. Probably the delayed surgical procedure is especially harmful to younger patients and elderly who are in the category of low preoperative risk (15). Meta-analysis by Simunovic et al. (2010) and Khan et al. (2009) analyzes the relation between delay of surgery with mortality and reach the conclusion that early surgical treatment results in a significant benefit in reducing mortality rates (16,17,18), shorter hospitalization and less postoperative complications (16,18), consistent with our results. Yet this analyses and observations are not sufficiently sensitive and powerful to discover what is the impact of early or delayed surgery on patients with poor general health status. Probably the inclusion of patients with poor general health in a group to which the surgery is performed within 48 hours cannot reveal the real effect of delaying the surgery on mortality. Thus, by controlling the results of mortality rates with additional factors such as specific chronic diseases, mobility and delirium before fracture, Orosz et al. (2004) reported that the surgery done in the first 24 hours after injury is no longer associated with a positive effect on mortality (19). The results of the Spanish population-based retrospective cohort study showed that the duration of the period admission/surgery has no impact on hospital mortality when the multivariate analysis taking into account age, gender and severity of associated chronic diseases (20). Also some studies done in the last century declare a lower mortality rate if the surgical treatment of hip fractures was postponed due to stabilization of the general health state, thus concluding that sicker patients may benefit from delayed surgery (21,22). This is confirmed by the results of the working group discussions by Moroni et al. (2011) when they pointed out that the surgery should be done within the first 24-48 hours after admission and that for the patients who have more than three comorbidities must be preoperatively optimized the general health status (23). However analysis of this and previous relevant studies shows methodological limitations, including lack of adequate statistical power and inadequate adjustment of known and potentially unknown preoperative factors. Weller et al. (2005) suppose that it is necessary to include more than 5,000 patients with adjustment of other risk factors, in order to detect statistical ratio of 1.2 (80% power, $\alpha = 0.05$) in order to favor earlier surgery. Even more patients would be required if adjustments for confounding were to be made (24).

Precise relations of characteristics before the injury such as age, sex, comorbidity, ASA status, type of fracture and mobility with the perioperative complications of geriatric patients after hip fracture is still unclear. Our study showed that all patients who had lethal outcome had ASA 3 and ASA 4 score. Although the ASA status of patients is used to assess the clinical complexity of the patient it is occasionally been criticized for its subjective nature and seems as a significant

predictor that a patient will have at least one perioperative complication or an increased mortality rate (25,26). The results which show an increased mortality rate among men with hip fractures are consistent with results of previous studies that have been published a higher rate of mortality among men after hip fracture, compared to women (12,27-30). Although the possible causes of increased mortality after hip fracture which men are facing compared to women are still unclear, increased mortality of men can be explained by the interaction of fracture with primary comorbid status, because survival among men with hip fractures is less than in the control a group of the same age with any level of comorbidity (31). It is supposed there are also some gender differences in terms of the tendency of men to infectious complications, particularly pneumonia and septicemia which could contribute to difference in risk (32).

Although the results of our study showed that the time of operative treatment was significantly associated with increased mortality of geriatric patients six months after surgical treatment of hip fractures, we were unable to prove a direct connection without exclusion of patient's characteristics before the injury. The high mortality rate of these patients is probably a common consequence of trauma and the impact of major surgical interventions in geriatric patients with low physiological potential.

6. Conclusion

The risk of mortality in hip fracture patients was almost three fold higher than that in relation to the general population of the same age group. Patients who underwent surgery within 48 hours after the injuries have a significantly lower rate of mortality and morbidity compared to patients whose surgery was postponed for more than 48 hours after the injury, suggesting that elderly patients with hip fractures should be operated on as soon as their medical condition permits.

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Effects of ephedrine and phenylephrine on the correction of hypotension in the cesarean sections under subarachnoid anesthesia

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Abstract

Purpose: To observe the effects of ephedrine and phenylephrine on the correction of hypotension in the cesarean sections under subarachnoid anesthesia.

Methods: 120 primiparas who received cesarean section in our hospital from March 2010 to June 2012 and used pressor drugs intra-operatively were selected and randomly divided into an ephedrine group (n = 60, 8 mg/ml) and a phenylephrine group (n = 60, 100 μg/ml). Before and after anesthesia as well as to the end of surgery, the dosages of the two drugs, maternal blood pressure, heart rate and respiratory changes were recorded. At the same time, maternal nausea and vomiting were also recorded. At the moment when the baby was delivered, the maternal arterial blood, umbilical arterial and umbilical venous blood gases were analyzed and the concentrations of epinephrine, norepinephrine, phenylephrine and ephedrine were measured. The Apgar scores of newborns (1 min and 5 min) were estimated.

Results: There were no significant differences in the equivalent usages of the two drugs and maternal blood pressure and respiratory changes. The neonatal 1 min- and 5 min- Apgar scores had no statistically significant differences. The maternal heart rate, maternal nausea and vomiting of the phenylephrine group changed less significantly than those of the ephedrine group ($P < 0.05$). The umbilical arterial and umbilical venous pH and base excess of the ephedrine group were significantly lower than those of the phenylephrine group ($P < 0.05$), while the umbilical artery PCO_2 and umbilical vein PO_2 were significantly higher in the ephedrine group than those in the phenylephrine group ($P < 0.05$). The concentrations of umbilical arterial epinephrine and norepinephrine were significantly higher in the ephedrine group than those in the phenylephrine group ($P < 0.05$).

Conclusion: Phenylephrine outweighs ephedrine in correcting maternal hypotension during cesarean section under subarachnoid anesthesia.

Key words: Epinephrine, phenylephrine, subarachnoid anesthesia, cesarean section.

Introduction

Subarachnoid anesthesia, referred to as spinal anesthesia, is an anesthesia method commonly used in cesarean section by injecting local anesthetic drugs into subarachnoid space through lumbar intervertebral space to block the involved nerve root [1]. For its advantages of full blockade and good muscle relaxation, spinal anesthesia is widely welcomed by obstetricians and anesthesiologists, which is also conducive to postoperative analgesia. Its disadvantage is that peripheral vasodilatation and relaxed and increased uterine will compress the inferior vena cava to greatly reduce returned blood volume after most puerperas are subjected to spinal anesthesia, resulting in hypotension. The method often adopted to correct hypotension is to lie puerpera in a left lateral position with rapid intravenous infusion of a large quantity of crystalloid solution and vasoactive agents [2]. Ephedrine, as an adrenomimetic drug, can excite sympathetic nerves, relax bronchial smooth muscle and contract blood vessels with a significant effect in central excitation, and it functions longer than epinephrine [3]. Phenylephrine mainly excites α receptors, the pressor effect of which is weaker and more enduring than norepinephrine (1 hour for intramuscular injection and 20 minutes for intravenous injection). Phenylephrine is less toxic and can reflexively stimulate the vagus nerves by contracting blood vessels and increas-

ing blood pressure to lower heart rate, which is not usually used in surgery [4]. This study aims to observe whether phenylephrine is more advantageous than ephedrine in the safety and efficacy in correcting hypotension during cesarean section under spinal anesthesia.

Materials and Methods

General information

120 primiparas who received cesarean section in our hospital from March 2010 to June 2012 and used pressor drugs intra-operatively were selected as the study object, all of whom were aged between 22 and 30 years old and pregnant for 38 to 42 weeks without pregnancy complications, and their fetuses were single and in a head position. All the objects had signed informed consent after communications. The puerperas were randomly divided into two groups (n=60): an ephedrine group (8 mg/ml) and a phenylephrine group (100 μg/ml).

Anesthesia methods [5]

After successful puncture in open vein and right radial artery, the puerperas were connected to MP20 monitor, infused with 20 ml/kg Ringer's solution within 15 min, and then conducted L3~4 epidural puncture with a 18 G epidural needle in the left lateral position. After successful puncture, spinal anesthesia puncture was performed with an inserting needle. After seeing the outflow of cerebrospinal fluid, 2 ml of 0.5% bupivacaine (10 mg) was infused into the subarachnoid space (needle oblique face up to head) in 60 s. Then the puerperas were laid supine, and inhaled with oxygen at 3 L/min via nasal cannula simultaneously to maintain the oxygen saturation (SpO2 ≥) 99%. When the maternal blood pressure was lower than 30% of the basal one, pre-prepared ephedrine or phenylephrine was given to the objects according to the numbers of odd and even until the maternal blood pressure returned to normal. The doses of the two drugs, maternal blood pressure, heart rate and respiratory changes were recorded before and after anesthesia and to the end of surgery. In the meantime, maternal nausea and vomiting were also recorded. Whether there were anesthesia-related complications during hospitalization was recorded. Immediately after fetus delivery, 4 ml of maternal arterial blood, 4 ml of neonatal umbilical arterial and 4 ml of umbilical venous bloods were drawn respectively and added heparin as anticoagulant, of which 2 ml was taken for blood gas analysis, and the other 2 ml for measuring the concentrations of ephedrine, epinephrine and norepinephrine in the ephedrine group, and the concentrations of phenylephrine, epinephrine and norepinephrine in the phenylephrine group. 1 min and 5 min Apgar scorings were conducted for the newborns.

Apparatuses and detection methods

Blood gas analysis was conducted by Radiometer ABL80 blood gas analyzer (Radiometer, Denmark) immediately after the extraction of blood samples. The other apparatuses include Guochuang PC2001 high-performance liquid chromatograph (Hefei Guochuang) and diode array UV detector (Nanjing Xianlin Company).

Plasma concentration determination

Blood samples were centrifuged to retrieve the supernatant which was then dried at low temperature and stored.

Epinephrine content determination

Appropriate amount of epinephrine was weighed precisely and transferred into a 25 ml volumetric flask, to which was added acetic acid solution that was diluted to scale. Then 20 μl of the solution was precisely transferred into the liquid chromatograph to record the chromatogram. Another epinephrine reference was accurately weighted, to which was added acetic acid solution until 0.12 mg/ml that was measured with the same method. The results were calculated by peak area according to the external standard method.

Determination of norepinephrine and phenylephrine contents

The norepinephrine sample was precisely measured and placed into a 25 ml volumetric flask, to which was added acetic acid solution that was diluted to scale and shaken well. Then 20 μl of the solution was precisely taken into the liquid chromatograph to record the chromatogram. Another norepinephrine reference was taken appropriately for accurate weight, to which was added acetic acid

solution until 0.16 mg/ml that was measured with the same method. The results were calculated by peak area according to the external standard method.

Determination of ephedrine content

The sample was precisely measured and evaporated to dryness on a water bath, and dried at 105 ℃ for 1 h, cooled to room temperature, to which were successively added 10 ml of glacial acetic acid, 5 ml of acetic acid mercury test solution, 2 ml of acetic anhydride and 1 drop of crystal violet indicator solution. The solution was then titrated with perchloric acid titration solution (0.1 mol/L) to jade green, and finally the titration results were corrected with the blank. Per 1 ml perchloric acid titration solution (0.1 mol/L) was equivalent to 20.17 mg hydrochloric acid ephedrine.

Statistical analysis

All the data were analyzed by SPSS 17.0 and expressed as ($\bar{x}\pm s$). The numeration data were expressed as case number or percentage. The groups were compared by the paired t test. The measurement data were compared by the χ^2 test. P<0.05 was considered statistically significant.

Results

120 puerperas were selected to complete the whole test. There were no statistical differences between the two groups in age, height, weight, intra-operative level of anesthesia and the time from the start of anesthesia to fetal delivery (Table 1).

The preoperative systolic blood pressure, total amount of vasopressor drugs and liquid intake had no significant differences between the two groups. The neonatal 1 min and 5 min Apgar scores [6] were not significantly different. 8 puerperas in the phenylephrine group underwent bradycardia, which was significantly more than the number in the ephedrine group (P <0.05). There were 16 cases of maternal nausea or vomiting in the ephedrine group, and 2 cases of nausea in the phenylephrine group, between which the difference was significantly significant (P <0.05) (Table 2).

No significant difference was detected in the arterial blood gas analysis between the two groups. The neonatal umbilical arterial and umbilical venous pH and base excess in the ephedrine group were significantly lower than those in the phenylephrine group (P <0.05), but the umbilical artery PCO_2 and the umbilical vein PO_2 were significantly higher in the ephedrine group than those in the phenylephrine group (P <0. 05) (Table 3).

The concentrations of umbilical arterial epinephrine and norepinephrine in the ephedrine group were significantly higher than those in the phenylephrine group (P <0. 05) (Table 4). No lower extremity hypoesthesia or hypotonia, bladder dysfunction and other anesthesia-related com-

Table 1. General information of the puerperas ($\bar{x}\pm s$)

Item		Ephedrine (n=60)	Phenylephrine (n=60)
Age (years old)		25.5±4.2	24.9±4.6
Body weight (kg)		64.3±10.6	65.2±10.8
Height (cm)		160.2±7.9	159.7±8.3
Highest blocking level	Range	T3~T6	T4~T7
	Average	T5	T6
Time from anesthesia start to fetal delivery (min)		19.8±2.6	19.9±2.3

Table 2. Doses and blood flow dynamic changes ($\bar{x}\pm s$)

Item	Ephedrine (n=60)	Phenylephrine (n=60)	P value
Preoperative systolic blood pressure (mmHg)	125±8	126±7	0.47
Minimum systolic blood pressure (mmHg)	86±6	85±7	0.41
Minimum heart rate (bpm)	71±5	57±5	<0.05
Pressor drug dose (ml)	4±1	3±1	0.67
Total fluid amount (ml)	1921±320	1852±332	0.46
Bradycardia (case)	1	8	<0.05
Nausea and vomiting (case)	16	2	<0.05

Table 3. Blood gas analysis results ($\bar{x}\pm s$)

Item		Ephedrine	Phenylephrine	P value
Puerperal arterial blood	pH	7.40±0.02	7.41±0.02	0.26
	PCO_2	34±2	34±3	0.32
	PO_2	110±11	111±10	0.57
	BE	-2.25±0.61	-2.26±0.65	0.73
Umbilical venous blood	pH	7.31±0.03	7.35±0.02	<0.05
	PCO_2	46±5	46±6	0.47
	PO_2	28±3	26±2	<0.05
	BE	-3.79±1.83	-1.42±0.92	<0.05
Umbilical arterial blood	pH	7.26±0.03	7.34±0.03	<0.05
	PCO_2	58±6	49±7	<0.05
	PO_2	23±2	23±2	0.55
	BE	-4.52±3.08	-1.66±1.35	<0.05

Table 4. Concentrations of epinephrine, norepinephrine, phenylephrine and ephedrine ($\bar{x}\pm s$)

Item		Ephedrine	Phenylephrine	P value
Puerperal arterial blood	Epinephrine (ng/ml)	46.3±22.6	32.5±21.7	P<0.05
	Norepinephrine (pg/ml)	289.2±72.9	116.4±37.7	P<0.05
	Phenylephrine (pg/ml)	-	7.6±2.5	
	Ephedrine (ng/ml)	351.1±63.4	-	
Umbilical venous blood	Epinephrine (ng/ml)	132.7±50.3	95.8±44.9	P<0.05
	Norepinephrine (pg/ml)	1622.4±843.9	453.5±179.2	P<0.05
	Phenylephrine (pg/ml)	-	1.2±0.5	
	Ephedrine (ng/ml)	421.0±102.3	-	
Umbilical arterial blood	Epinephrine (ng/ml)	707.8±242.9	512.6±240.1	P<0.05
	Norepinephrine (pg/ml)	5619.7±2279.2	2089.7±682.3	P<0.05
	Phenylephrine (pg/ml)	-	1.0±0.3	
	Ephedrine (ng/ml)	360.2±103.7	-	

plications were observed in all the selected cases during the follow-up 24 h and 72 h after the end of surgery and upon discharge.

Discussion

After puerperas undergo spinal anesthesia, peripheral vasodilatation and relaxed and increased uterine will compress the inferior vena cava to greatly reduce returned blood volume, thereby leading to hypotension. Most puerperas lie in a left lateral position at 30° with intravenous infusion, which cannot maintain the original blood pressure, which can commonly be treated with ephedrine [7]. Ephedrine can directly excite α1, β1 and β2 receptors, and can also facilitate norepinephrine nerve endings to release norepinephrine to act indirectly so as to elevate blood pressure [8]. It is disadvantageous in increasing heart rate, elevating myocardial oxygen consumption, and producing drug resistance rapidly. The elevated vagal tone essentially accounts for nausea and vomiting. β adrenergic agonists can also lead to vagus nerves reflex, thereby reducing cardiac preload and peripheral vascular resistance, so as to cause nausea and vomiting that cannot be prevented by 5-HT receptor antagonists [9]. Phenylephrine directly excites α receptors, its stimulating action on α1 receptor more evidently than that on α2 receptor. After intravenous injection, it can elevate the systolic and diastolic blood pressure, and reflexively decrease heart rate. Once there is a slight reduction in cardiac output, peripheral resistance will significantly increase, so that pulmonary artery pressure rises, whereas coronary blood flow increases [10]. Phenylephrine may increase peripheral vascular resistance, effectively suppress adverse stimulation inducd by vagal reflex and β adrenergic receptor stimulation and increase cardiac preload

[11]. Studies have found that phenylephrine is more effective in maintaining blood pressure with a lower incidence of hypotension and rescue event than ephedrine [12]. This study also found that phenylephrine significantly reduced the side effects on the maternal side such as nausea, vomiting, and etc. [13]. In short, phenylephrine not only possesses the advantages of ephedrine in maintaining blood pressure, but also can overcome the disadvantages in heart rate increase, myocardial oxygen consumption increase, nausea, vomiting, and etc. [14]. The perioperative impact of drugs on fetus must be considered [15]. Most vasoactive drugs can enter embryo blood circulation through the placental barrier, but it exerts less effects on the fetus in the presence of lower amount [16,17]. Umbilical venous pH value can reflect the degree of fetal acidemia and indirectly reflect fetal hypoxia [18].

This study shows that more ephedrine passes through the placental barrier, so that the umbilical venous pH was lower and the early metabolism and redistribution in the fetus were slower than those administered with phenylephrine [19]. In the phenylephrine group, neonatal umbilical arterial and venous pH were higher and the umbilical arteriovenous epinephrine and norepinephrine contents were significantly lower than those in the ephedrine group, confirming that ephedrine can be converted to epinephrine and norepinephrine or promote their secretions [20]. Nevertheless, umbilical venous PO_2 and umbilical arterial PCO_2 in the ephedrine group were higher than those in the phenylephrine group, in which the former might be relevant with the phenylephrine's contraction of the uterus, and the latter might result from the excessive epinephrine and norepinephrine [21]. Ephedrine increases the basal metabolism of fetus by exciting fetal β receptors, leading to fetal acidemia [22]. Therefore, compared to ephedrine, phenylephrine can enable the fetus to maintain a relatively stable acid-base balance.

In summary, phenylephrine has many advantages in increasing blood pressure, and will not increase catecholamines in neonatal body with a small amount through the placental barrier, which exerts little impact on neonate. Thus, phenylephrine outweighs ephedrine in correcting maternal hypotension during the cesarean section under subarachnoid anesthesia.

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Evaluation of breast lesions by magnetic resonance imaging

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Abstract

Introduction: During the last three decades, MRI gained more and more clinical application and from the experimental technique became mandatory clinical modality. Magnetic resonance imaging is the method with maximum sensitivity in the detection of breast cancer.

Goal: The goal was to correlate MRI, mammography and mamosonography assessment of breast lesions.

Materials and Methods: The study was prospective, clinical-radiological which included 33 patients. The patient underwent a MRI breast examinations by MR scanner Siemens Avanto 1.5 T with breast coils. Used are both FLASH and 3D axial sequences, one series before and eighth series after administration of gadolinium contrast. Evaluated are the kinetic and morphological parameters by the Fisher protocole. Indications for MRI examination were: preoperative stegeing for breast cancer, benign lesions of intermediate characteristics BI-RADS 3 categories and estimate of silicone implant. For all patients was previously made clinical, mammography and ultrasound examination and core biopsy for 22 patients.

Results: Breast cancer was diagnosed in 18 patients. According to Fisher protocol scores ranged from 5 to 8. Detection of contralateral breast cancer, which was not diagnosed by mammography or ultrasound is found in 1 case. Benign lesions by PH are verified in 4 patients which by Fisher protocol had scores of 0-2. Diameter of cancer is larger in cese of MRI evaluation in comparison with ultrasound and mammography findings by 0.4-2.2 cm. Diameter of benign lesions on MRI findings was larger by 1.6 to 7.2 cm in comparison with mammography and ultrasound findings.

Conclusion: Mammography, Ultrasound and MRI are complementary methods in the diagnosis of breast diseases. MR mammography is the method of high sensitivity in the detection of breast cancer and superior in morphological characterization of breast lesions except calcifications. MRI in comparison with mammography and ultrasound is significantly more sensitive in detecting tumor size, multiplicity and multicentricity. This makes it the method of great importance in the preoperative staging of breast cancer.

Key words: breast cancer, MRI, mammography, ultrasound.

Introduction

During the last three decades, MRI gained more and more clinical application and from the experimental technique became mandatory clinical modality. Mammography is still the most commonly used method in the screening and in the preoperative breast cancer assessment. However MRI is becoming increasingly important modality in the detection and preoperative assessment of breast cancer (1,2,3). The high sensitivity of MRI in the detection of breast cancer makes possible diagnosis of breast cancer with a diameter of 3 mm and with a high degree of accuracy. It is reasonable to expect that breast MRI can significantly reduce mortality from breast cancer. Increasing quality of diagnostic information obtained by MRI justifies a higher cost of this method in comparison with mammography and ultrasound (2).

Magnetic resonance imaging is the method with maximum sensitivity in the detection of breast cancer. The specificity of MRI significantly increases by the knowingly use of all the data provided by MR mammography, thus reducing unnecessary biopsies and excisions. In comparison with conventional methods such as mammography and ultrasound, magnetic resonance imaging sensitivity is higher and allows, not only morphological, but

also functional characterization of lesions. Still, use of all diagnostic modalities, mammography, ultrasound and MRI, provides the largest amount of information, and thus the diagnostic evaluation of lesions is of the greatest accuracy.The most important indications for MRI are preoperative local staging, differentiation of scar tissue and recurrent cancer, screening for high-risk populations, unknown primary cancer, evaluation of implants, problem cases unresolved by conventional diagnostic methods: mammography and ultrasound assessment of therapeutic response to neoadjuvant chemotherapy. The necessary technical conditions for performing this method are: a magnetic field of 1-3 Tesla, bilateral breast coils, appropriate protocol and postprocesing.

Breast cancer is a malignant disease with the highest incidence and mortality in the female population of Europe (4). Demographic indicators show a trend of continuous increase in number of women developing breast cancer, which is why it becomes important public health problem. Early detection of breast cancer by the methods of choice, efficient dignostic algorithms and optimal therapeutic approach are the basis of its successful treatment. The primary objective is to reduce the mortality rate of women from this disease and provide them better quality of life. It is known that the treatment outcome prognosis depends on the histopathologic type, size and stage of the tumor at time of diagnosis and detection of cancer in the early stages has favorable treatment outcome (3,5).

Goal

The goal of this study is to correlate MRI, mammography and mammosonography assessment of breast lesions.

Material and methods

Material

This study is prospective, clinical-radiologic, which included 33 patients, for diagnostic evaluation of breast lesions. All patients underwent MRI examination of the breast and previously, clinical examination, mammography, mammosonography and 22 patients core biopsy.

Methods

Mammography included standard MLO, CC projections, and depending on the lesion also additional images. Equipment used was Mammomat 3000 New by Siemens. Mammosonography included conventional color/power Doppler ultrasound. Used is 7.5 to 12 MHz linear probe on Voluson 730 GE, and Siemens Sonoline G50. All lesions are shown in sagittal and transverse plane with the assessment of vascularization. MRI examination is performed by machine Siemens Avanto 1.5 T with breast coils. The protocol included axial T 2 W sequences with thickness of 3 mm, axial 3D FLASH sequence with thickness of 0.9 mm. One series before and 8 after administration of gadolinium contrast agent. Postprocesing included subtraction, analysis of IS curve and MIP reconstruction. To perform a biopsy was used BARD Biopsy Magnum gun with a 14 G needle of 10cm length. During the MRI assessment of lesions is used BI-RADS and the Fisher multifactorial evaluation protocol with the kinetic and morphological parameters.

Morphological criteria with scoring were:

1. Lesions shape (0-round, 0-polygonal, 0-linear, 1-branching, 1-spiculated);
2. Lesion margins (0-well-defined, 1-indistinct);
3. Enchancing pattern (0-homogeneous, inhomogeneous-1, 0-septated, 2-ring form).

Dynamic parameters with scoring:

4. The initial singnal increase (<50%-0, from 0.50 to 100%-1, >100%-2)
5. Postinitial signal increase (steady increase-0, plateau-1, wash-out-2).

To Fisher scoring corresponds the following MRM-BIRADS categories: 1 (0-1); 2 (2); 3 (3); 4 (4-5); 5 (6-8).

Results

From a baseline of 33 patients, 22 (66.7%) underwent mammography, mammosonography, MRI and core biopsy with B-IRADS 3,4 and 5 categories, and 11 (33,3%) of patients underwent mammography, mammosonography and MRI of the breasts with BIRADS 2 and 3 categories (Table 1). Indications for breast MRI – MR mammography was: unclear cases in 4 (17.4%) of patients, evaluation of breast cancer before surgery in 18

(78.3%) of patients, and evaluation of the silicone breast implant in 1 (4.3%) of cases (Table 2).

Table 1. Diagnostic methods

	N	%
Clinical examination	33	100.0
Mammography	33	100.0
Mammosonography	33	100.0
MRI	33	100.0
Core biopsy	22	66.7
Total sample	33	100.0

Table 2. Indications for breast MRI – MR mammography

	N	%
Unclear diagnosis	4	17.4
Evaluation of breast cancer before surgery	18	78.3
Evaluation of the breast silicone implant	1	4.3
Total sample	23	100.0

Patients with BI-RADS 3, BI-RADS 4 and BI-RADS 5 lesions category (22 patients) after mammography, ultrasound and MRI underwent core biopsy.

Patients with BI-RADS 2 and BI-RADS 3 lesions category (11 patients) after mammography and ultrasound underwent MRI. Afterwards all lesion were evaluated as BI-RADS 2 and its benign status was confirmed by two years follow up

Table 3. BI-RADS categories

	N	%
BI-RADS 3, 4 and 5	22	66,7
BI-RADS 2 and 3	11	33,3
Total sample	33	100.0

From 22 patients by application of mammography, mammosonography, breast MRI and core biopsy with BIRADS 3,4 and 5 in 18 (81.8%) patients was diagnosed breast carcinoma, and in (18.2%) cases benign lesions (Table 4).

Table 4. Histopathology findings

		N	%
Malignant tumors (N=18, 81.8%)	Ductal invasive	13	59.1
	Lobular invasive	4	18.2
	Ductal and lobular	1	4.5
Benign tumors (N=4, 18.2%)	Giant fibro adenoma	1	4.5
	Fibrocystic mastopathy	1	4.5
	Complex fibroadenoma	1	4.5
	Hamartomas	1	4.5
Total		22	100.0

Malignant tumors

Tumor size in these patients was in range from 1.9cm to 5.2cm.

Diameter of malignant tumors in our sample evaluated by MRI was in range from 1.9 cm to 5.4 cm.

Diameter of malignant tumors evaluated by mammography was in range from 1.4cm to 3.6 cm.

Diameter of malignant tumors in our sample evaluated by ultrasound was in range from 1.5 cm to 3.2 cm.

Mean diameter of tumor mass was 3.5±0.9

Mean diameter of tumor mass evaluated by MRI was 3.6±0.9.

Mean diameter of tumor mass evaluated by mammography was 2.5±0.6.

Mean diameter of tumor mass evaluated by ultrasound was 2.5±0.5.

Difference in evaluation of real tumor mass diameter (PH substrate) and MRI diameter was in range from -0.2 cm to 0.5 cm, mean 0.111±0.17. True size of tumor mass (PH substrate) equal to MRI dimensions of tumor mass was recorded in 4 cases. True size of tumor mass (PH substrate) smaller than MRI dimensions was recorded in 11 cases, and larger in 3 cases.

Difference in evaluation of real tumor mass diameter (PH substrate) and mammography diameter was in range from -2.1 cm to 0.4 cm, mean -0.956±0.5. Equal size of tumor mass (PH substrate) to mammography dimension was not recorded, as well as smaller one. True size of tumor mass (PH substrate) larger than mammography dimension was recorded in all 18 cases.

Difference in evaluation of real tumor mass diameter (PH substrate) and ultrasound diameter was in range from 2.6 cm to -0.2 cm, mean -1.03±0.7. Equal size, as well as size of tumor mass (PH substrate) smaller than ultrasound size was not recorded. True size of tumor mass (PH substrate) larger than ultrasound dimension was recorded in all 18 cases.

Benigne lesions

Benign lesions diameter in this sample was in range from 3.2 cm to 25 cm.

Diameter of benign lesions in our sample evaluated by MRI was in range from 3.5 cm to 24 cm.

Diameter of benign lesions evaluated by mammography was in range from 1.9 cm to 19 cm.

Diameter of benign lesions in our sample evaluated by ultrasound was in range from 2.9 cm to 17 cm.

Mean diameter of benign tumor mass was 14.3±12.4.

Mean diameter of benign tumor mass evaluated by MRI was 13.9±11.7.

Mean diameter of benign tumor mass evaluated by mammography was 6.7±8.2.

Mean diameter of benign tumor mass evaluated by ultrasound was 10.02±8.1.

Difference in evaluation of real tumor mass diameter (PH substrate) and MRI diameter was in range from -1 cm to 0.3 cm, mean -0.5±0.7. True size of tumor mass (PH substrate) equal to MRI dimensions of tumor mass was not recorded. True size of tumor mass (PH substrate) smaller than MRI dimensions was recorded in 1 case, and larger in 3 cases.

Difference in evaluation of real tumor mass diameter (PH substrate) and mammography diameter was in range from -23.1 cm to -0.4 cm, mean -7.6±10.6. Equal size of tumor mass (PH substrate) to mammography dimension was not recorded, as well as smaller one. True size of tumor mass (PH substrate) larger than mammography dimension was recorded in all 4 cases.

Difference in evaluation of real tumor mass diameter (PH substrate) and mammography diameter was in range from -8 cm to -0.3 cm, mean -4.3±4.3. Equal size of tumor mass (PH substrate) to mammography dimension was not recorded, as well as smaller one. True size of tumor mass (PH substrate) larger than mammography dimension was recorded in all 4 cases.

From a total of 18 patients which by PH analysis was confirmed breast cancer, by Fischer protocol scoring was 5-8 points, MRM BIRADS category 4 and 5, from that number 1 multicentric, 1 multifocal, 1 bilateral cancer without mammography and ultrasound detection. PH benign lesions were diagnosed in 4 patients, according to Fischer protocol scoring of 3-4 points. Benign lesions in 11 patients according to Fisher protocol scored 0-2 points, MRM BIRADS 1 and 2 (Table 6).

Table 6. Fischer protocol

	N	%
5-8 points PH breast cancer	18	54.5
3-4 points PH benign lesions	4	12.1
0-2 points Benign lesions	11	33.3
Total	33	100.0

Table 5. Size of tumor mass: evaluated by mammography, ultrasound, MRI and tumor size

		N	Mean	Std. Deviation	Minimum	Maximum
Mammography size (cm)	Benign	4	6,675	8,2306	1,9	19,0
	Malignant	18	2,533	,5881	1,4	3,6
Difference of mammography and tumor size (cm)	Benign	4	-7,625	10,6177	-23,1	-,4
	Malignant	18	-,956	,5349	-2,1	-,4
Difference of mammography and MRI size (cm)	Benign	4	-7,175	10,1454	-22,1	-,7
	Malignant	18	-1,067	,5499	-2,3	-,3
Ultrasound size (cm)	Benign	4	10,025	8,0550	2,9	17,0
	Malignant	18	2,461	,5282	1,5	3,2
Difference of ultrasound and tumor size (cm)	Benign	4	-4,275	4,3061	-8,0	-,3
	Malignant	18	-1,028	,7323	-2,6	-,2
Difference of ultrasound and MRI size (cm)	Benign	4	-3,825	3,6664	-7,0	-,6
	Malignant	18	-1,139	,7800	-2,8	-,2
MRI size (cm)	Benign	4	13,850	11,7213	3,5	24,0
	Malignant	18	3,600	,9598	1,9	5,4
Difference of MRI and tumor size (cm)	Benign	4	-,450	,6557	-1,0	,3
	Malignant	18	,111	,1711	-,2	,5
PHS size (cm)	Benign	4	14,300	12,3596	3,2	25,0
	Malignant	18	3,489	,9399	1,9	5,2

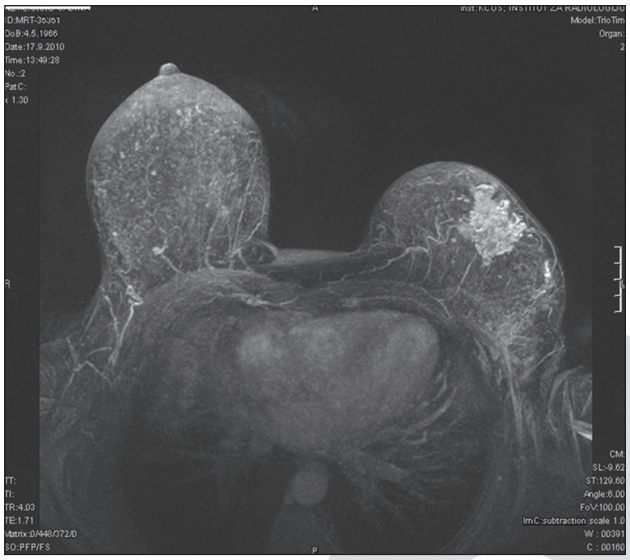

Figure 1. Preoperative assessment of ductal carcinoma

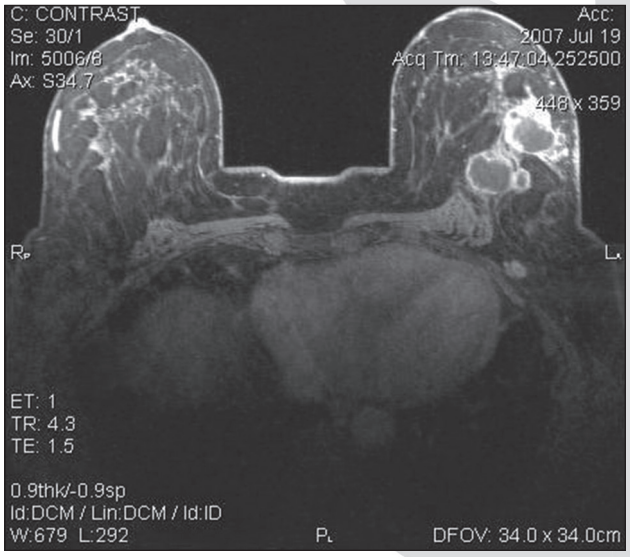

Figure 2. Preoperative assessment of lobular carcinoma

Discussion

A little-known fact is that the breast represents one of the earliest subjects of research in the field of MRI. Damadian in 1971, Mansfield in 1979 were trying to differentiate breast tumors. In the mid-eighties, by the development of FLASH sequences, J. Frahm and A. Haase, enables dynamic imaging and the use of paramagnetic contrast agents. The development of special breast coils, improves spatial resolution. Sylvia Heywang by introducing 3D FLASH sequences and MRI parallel imaging system optimizes MR mammography (2,3,4,5).

Breast MRI has increasing clinical application. In comparison with mammography and ultrasound it provides not only morphological information, but also functional characteristics of the lesion (tissue perfusion and of contrast media opacification kinetics (6,7).

Breast MRI has high sensitivity and low to moderate specificity. Technical problems: divarication in high temporal and spatial resolution (8).

Breast MRI shows the highest sensitivity in the detection of primary and recurrent invasive cancer. By improvement of MR technology, interpretation criteria, also improved the specificity and positive predictive value of this method (9).

Breast tumors may be solitary, well defined and then be easily detected by mammography and ultrasound. Tumor size estimated by mammography and ultrasound can be significantly smaller in comparison with MRI dimensions, particularly in case of tumors larger than 2 cm which is influencing surgergical procedures (10).These findigs correlate to our study. By comparing the findings of mammography, MRI and ultrasound in high-risk women, MRI results in greater number of biopsies but also detection of more cancers (10).

Unilateral cancer increases the risk of contralateral cancer. After an MRI examination, contralateral cancer is detected in 1up to 10% of patients who are not diagnosed by mammography and ultrasound (11). The sensitivity of MRI in the detection of contralateral cancers is 91%, with specificity of 88% (11). MRI is superior in assessing intraductal spread and tumor size. Tumor size of invasive cancer estimated by MRI corresponded to the dimensions of the pathological substrate in our study Multycentricity of the cancer, which represents one or more invasive focus within 4 cm and more from the primary tumor, is present in 20% of invasive cancers. Inadequate estimate of the tumor size, the failure to detect additional focuses leads to positive resection and therefore early disease relapse (12,13,14).

Conclusion

MR mammography, mammography and mamosonography are complementary methods in the diagnosis of breast lesions.

MR mammography is the method of high sensitivity in the detection of breast cancer.

MRM is superior in comparison with mammography and mamosonography to determine the morphological lesions characteristics, evaluation of tumor size, multiplicity, multicentricity and bilateral cancers.

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Efficiency of sildenafil monotherapy in benign prostatic hyperplasia

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Abstract

Summary: Very high incidence of clinical significant BPH (LUTS) and different therapeutic options of medical treatment have very high socioeconomic importance. There are few previously published papers that proved efficiency of PDE5 inhibitors in treatment of this medical problem. Still, more studies and longer follow up in this therapy approach are needed.

Objective: Changes in International Prostate Symptom Score (IPSS), post voiding residual (PVR) and maximal uroflow (Qmax) in BPH/LUTS patient after treatment with continuous, low dose of sildenafil.

Material and methods: Our research has been conducted as prospective, controlled, opened and randomized study, and involves 30 men with BPH/LUTS. Research has been conducted at Urology Department, Clinical Centre of Vojvodina in one year time frame (November 2011 till November 2012). All patients were >45 yrs old, IPSS was >3, Prostatic specific antigen (PSA) was <10, normal urinalysis (UA). Each patient was tested for Uroflow study – Qmax, and for post voiding residual (PVR).

Results: In observed group of patients at the end of our study we have seen statistically significant changes in all three parameters: mean IPSS value improved from 12,8 to 8,6 (32,8% change), mean PVR value has changed from 49,4 ml to 40,2 ml (18.6% change), and mean Qmax value changed from 11,8 ml/s to 12,8 ml/s (8,5% change).

Conclusion: Treatment of BPH/LUTS with continuous low dose of sildenafil (although there were not significantly changes in Qmax and PVR) looks like good treatment choice for patients with mild to moderate BPH/LUTS symptoms, especially in those patients, whose have concomitant ED. Place and role of this treatment approach still need to be determined.

Key words: Benignant prostatic hyperplasia, lower urinary tract symptoms, Qmax, post void residuum, international prostate symptom score, IPSS, sildenafil, PDE5 inhibitors.

Introduction

Benign prostatic hyperplasia symptoms (LUTS) results from: mechanical obstruction - intrusion of enlarged, hyperplasic prostatic tissue into urine pathway (bladder neck, urethra) and from dynamic obstruction - hyper stimulated adrenergic receptors of smooth muscle and collagen. For mechanical obstruction 5α-reductase inhibitors are drug of choice and for dynamic component of LUTS alpha blockers would be appropriate therapy.

Phosphodiesterase type 5 inhibitors (PDE5) inhibitors are group of drugs (sildenafil, tadalafil, vardanafil) that are widely used as a standard treatment for erectile dysfunction (ED) as well as pulmonary hypertension (only sildenafil and tadalafil). Nowadays PDE5 inhibitors are investigated for benignant prostatic hyperplasia (BHP)/LUTS treatment. Their mechanism of action would be by inhibition of autonomous stimulation of smooth muscle and collagen (inhibition of dynamic obstruction). PDE5 inhibitors act by link that involves the NO/cGMP pathway. Nitro oxide (NO) presents important non adrenergic, non cholinergic neurotransmitter in human body. It is involved in transmission of neuro signals in urinary tract as well. NO is produced from amino acid L arginine by influence of nitro oxide synthetasys (NOS). In human body they are classified by tissue origin as: neuronal NO synthetasys (nNOS), endothelial NO synthetasys (eNOS) and NO synthetasys of immune cells origin (iNOS). Once NO has been produced, it goes into cells and stimulates synthesis of cyclic guanosyn monophosphate (cGMP) by enzyme guanyl cyclase. cGMP acti-

vate protein kinase, ion channels, and cGMP binding phosphodiesterase which leads to relaxation of smooth muscles by decreasing concentration of intracellular Ca++ and desensibillation of contractile proteins (1)**.** Effects of cGMP are discontinued by PDE isoensyme which catalyze hydrolyzing of cGMP in its inactive form. PDE5 inhibits PDE isoenzyme and increase concentration and activity of intracellular cGMP and thus, decreases tonus of smooth muscle of detrusor, prostate and urethra. Until today there are 11 different PDE esterases discovered, among which are PDE esterases 4 and 5 predominant in transitional zone of human prostate, bladder and urethra (2,3). It is possible that NO is involved in micturition also by inhibition of reflex pathways in spinal cord (4).

Objective

Assessment of any changes in parameters of voiding: International prostate symptom score (IPSS), post voided residuum (PVR) and Qmax, assessed at the start of the study in patient without any treatment, in the middle of study and at the end of three month period during which patients were on sildenafil treatment.

Material and methods

Our research has been conducted as prospective, controlled, opened and randomized study, and involves 30 men with BPH/LUTS. Research has been conducted at Urology Department, Clinical Centre of Vojvodina in one year time frame (November 2011 till November 2012). All patients were >45 yrs old, IPSS was >3, PSA was <10, normal UA, DRE with no suspicion of cancer. To be included in study patient had to have no contraindication for using PDE5 inhibitors.

Assessment of parameters of voiding

Each subject, during first visit had physical examination (digitorectal examination, brief neurological examination), and IPSS questionary form was filled out. IPSS form has seven questions that are same as AUA-SI plus eight questions known as a bother score, which is designed to estimate disease specific quality of life (QoL). Each question brings 0-5 points, minimal number of points would be 0 and maximal number of points would be 35. Based on first seven questions and severity of patients symptoms all patients would be classified in three groups: first group - patients with mild symptoms (0-7 points), second group - patients with moderate symptoms (8-19 points) and third group – patients with severe symptoms (20-35 points). Each subject had to have IPSS at least 3 or more to qualify for study. Once patients had been qualified for study, assessment of Q max and PVR would be performed. For assessment of Qmax, Uroflow study had to be done. That study would be done on device Solar urodynamic machine – Medical measurements systems (the Netherlands). Each subject was required to void at least 150 ml of urine to have valid uroflow study and Q max value determined. Patient would be alone in examination room with activated Uroflow device and would void in standing position with comfort height level of urine baker (if a baker is too high, it would be uncomfortable for patient to void and results of the study would not be valid). Once patient has finished voiding, immediately PVR would be determined. PVR would be determined by the Ultrasound – SIEMENS – Sonoline Prima with abdominal ultrasound probe of 3,5 MHz. After all examination and tests were done patient would be given supply of sildenafil, enough for six week dosing of 1x25 mg – up to the next control. At the first control after six weeks, since the treatment was started, set of the same procedures, in the same manner would be done again (IPSS, PVR and Qmax). Patient would be again provided with six week supply of sildenafil, at dose level of 1x25 mg per day. After second six weeks cycle, patient would come for second and the last control and again set of the same procedures, in the same manner would be done again (IPSS, PVR and Qmax).

Result

Table 1. Collected mean values for International Prostate Symptom Score (IPSS), post voiding residual (PVR) and maximal uroflow (Qmax) for all 30 patients at the beginning of study

30 patients	PVR in ml	Qmax ml/s	IPSS value
Values at the beginning of study	49,4	11,8	12,8

Table 2. Shows collected mean values for International Prostate Symptom Score (IPSS), post voiding residual (PVR) and maximal uroflow (Qmax) of all patients at first control (six weeks upon starting sildenafil treatment)

30 patients	PVR in ml	Qmax ml/s	IPSS value
Values after six weeks of sildenafil	41,0	12,6	9,0

Table 3. Presents mean values for International Prostate Symptom Score (IPSS), post voiding residual (PVR) and maximal uroflow (Qmax) for all patients at the end of study (12 weeks after sildenafil treatment had been started)

30 patients	PVR in ml	Qmax ml/s	IPSS value
Values after twelve weeks of sildenafil	40,2	12,8	8,6

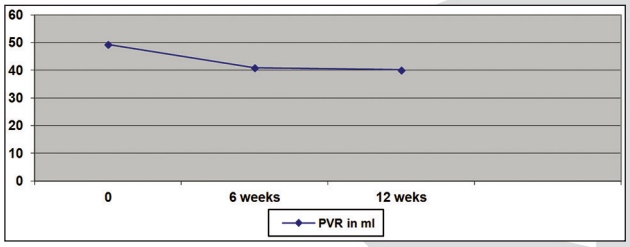

Chart 1. Represents changes in mean values of International Prostate Symptom Score (IPSS)

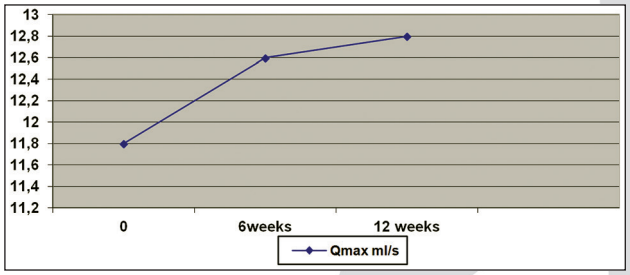

Chart 2. Presents changes in mean values of PVR

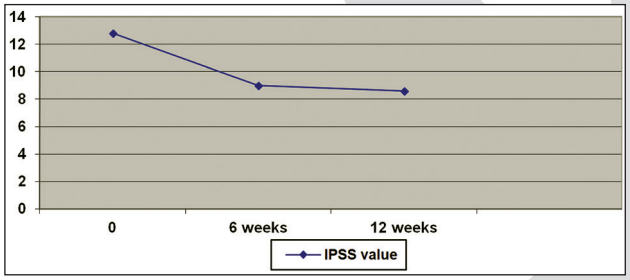

Chart 3. Presents changes in mean values of maximal uroflow (Qmax) after six and twelve weeks of sildenafil therapy

Discussion

BPH results by enlargement of prostate and can lead to lowering of urinary flow from urinary bladder. BPH is considered to be normal part of man aging and it is hormonally dependent of testosterone and dyhidrotestosterone (DHT) production. It is supposed that 50% of all man of age 60, has histological form of BPH. This number goes up to 90% at 85 year of life (5).

Lately (October 2011) use of PDE5 inhibitors for BPH/LUTS treatment became officially FDA approved in the USA. Still, there are contradictory data of efficiency level of BPH/LUTS treatment by PDE5.

In our study we have found that mean IPSS improved from 12,8 to 9,0 (29,6% change) and 8,6 (32,8% change), at the beginning of study, at first control (six weeks on sildenafil treatment) and at second/last control (after 12 weeks on sildenafil treatment). In most randomized, placebo controlled studies, that where conducted lately, efficiency of all three PDE5 inhibitors regarding IPSS, Qmax and PVR (6-15) changes were observed. Maximal time frame of studies was 12 weeks. In all of these studies, it was concluded that IPSS significantly improved, from 17% up to 35%. During PDE5 treatment, both groups of parameters – symptoms of bladder filling and voiding symptoms would equally decreased.

Regarding uroflow/Qmax changes, in our study we have found that mean Qmax value has changed: from 11,8 ml/s to 12,6 ml/s (6,8% change) and 12,8 ml/s (8,5% change), at the beginning of study, at first control (six weeks on sildenafil treatment) and at second/last control (after 12 weeks on sildenafil treatment). In most other studies (6-15) our findings were not confirmed: Qmax was not significantly different comparing to placebo.

In our study we have found change in mean PVR values before and during sildenafil treatment: 49.4 ml, 41.0 ml (17% change), 40.2 ml (18.6% change), at the beginning of study, at first control (six weeks on sildenafil treatment) and at second/last control (after 12 weeks on sildenafil

treatment). In almost all other studies there were not any significant changes of PVR (6-15).

But, there are few studies that proved contrary results: in two studies, after using 50 mg and 100 mg of sildenafil, Qmax increase was 3.7-4.3 mLs or 24-38% (16,17).

Also, there are three studies that compared efficiency of sildenafil mono therapy vs. sildenafil plus alpha blocker (alfuzosin or tamuslosin) combined therapy. There are three studies that compared efficiency of PDE5 inhibitors (sildenafil or tadalafil) with or without alpha blockers (alfuzosin or tamsulosin) (8, 11, and 12). These studies were conducted on limited number of patients and relatively short follow up time of six to twelve weeks. Combination of these drugs led to improvement in IPSS, Qmax and PVR in bigger numbers, then any drug from both groups given as a mono therapy. Although, difference comparing to PDE5 inhibitor or alpha blocker as a mono therapy was statistically significant only in one of three studies (11).

Conclusion

Treatment of BPH/LUTS with continuous low dose of sildenafil (although there were not significantly changes in Qmax and PVR) looks like good treatment choice for patients with mild to moderate BPH/LUTS symptoms, especially in those patients, whose have concomitant ED. Place and role of this treatment approach still need to be determined.

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Parathromone serum level before and after treatment for osteoporosis in patients with chronic spinal lesion

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Abstract

Introduction: Parathyroid hormone (PTH) plays an important role in bone metabolism. It is one of the causes of secondary osteoporosis in chronic phase of spinal cord lesion.

Objective: To compare PTH serum level before and after therapy with alendronate and kinesitherapeutic procedures in patients with chronic spinal cord lesion. (SCL).

Patients and methods: This prospective, longitudinal, clinical, comparative study included 36 patients with chronic SCL (24 or 67% males and 12 or 33% females) and secondary osteoporosis, diagnosed by DEXA densitometry of lumbar spine and proximal femur. The average patients age was 41.7±10.9 years and median of SCL occurrence was 12 years (IQR=11 to 13). Before and after 18 months therapeutic program for osteoporosis, which consisted of weekly alendronate tablet of 70 mg with daily calcium supplement of 500 mg and daily vitamin D3 of 400 i.u., and of kinesitherapeutic procedures, passive standing and exercises, levels of serum PTH was measured and compared. After completion of therapeutic program patients were divided in 4 groups for statistical analysis: (I) those who performed complete treatment program(*n=12*); (II) those who were not performing passive standing (*n=5*); (III) those who took only alendronate(*n=14*); (IV) those who discontinued performance of complete program after 12 months (*n=5*). A P value<0.05 was considered as significant. The following statistical tests were used: Kolmogorov-Smirnov test, Independent-Samples T Test, Wilcoxon Signed Rank Test.

Results: Before joining the therapeutic program 33% of patients had increased serum PTH level, average value of 63.16 pg/ml, and after performed program 20% of patients had increased level of PTH, average value of 49.74 pg /ml; (P<0.05). Increased PTH level correlated with worse DEXA densitometry finding of proximal femur. There was statistically significant difference in median PTH level in males before (Me=51.8 pg / ml, IQR 40.1 to 68.9) and after the treatment (Me=39.7 pg/ml, IQR =27.0 to 61.5) (Z=-2.257, P=0.023), and in female before (Me=53.9 pg/ml, IQR=42.6 to 79.3) and after the treatment. (Me=41.0 pg/ml; IQR=28.4 do 61.3) (Z=-2.981; P<0.01). In the age group of 20-44 years (*n=22*) median PTH level before treatment was 44.7 pg/ml (IQR=35.1 to 63.8), while after the treatment was 31.6 pg / ml (IQR=26.5 to 45.6), and the difference is highly statistically significant (Z=-3.685, P<0.001). In the age group ≥ 45 years (*n=14*), median levels of serum PTH was 64.3 pg/ml (IQR=50.2 to 99.9) before the treatment, while after the treatment was 64.8 pg/ml (IQR=44.3 to 75.8), and the difference was not statistically significant (Z=-1.287, P=0.217). The most significant decrease of serum PTH level had group I (t=3.37; P=0.04) and group IV (t=2.27; P=0.04).

Conclusion: Alendronate and kinesitherapeutic procedures led to a significant decrease of serum PTH levels in patients with chronic SCL and induced osteoporosis, especially in the age group of 20-44 years, and in patients who performed passive standing.

Key words: Spinal cord lesion, osteoporosis, parathormone.

Introduction

Certain complications of spinal lesion are not recognized because of their subclinical features, and one of them is osteoporosis induced by spinal lesion itself. According to classification it is *secondary* osteoporosis.

Osteoporosis induced by spinal lesion (SL) is, actually, inevitable complication, which begins to develop already tenth day after occurrence of spinal lesion, reaches its maximum after 4-6 months, then stabilizes after 12-16 months. It is found in 60-90% of patients with SL. (1)

Mechanism of spinal lesion induced osteoporosis is complex and still incompletely explained. Initially studied in the light of altered metabolism of calcium and hypercalcemia associated hypercalcuria and the loss of load. Only lately characteristics of this specific osteoporosis induced by SL were differentiated from bone loss caused by bed rest, staying in a vacuum or damage of peripheral motor neurons. Multifactor mechanism of its formation, both in acute and in chronic phase of SL, examines in detail only in the last ten years.

In addition to neurological changes caused by spinal cord lesion, especially interruption of pathways which are involved in bone innervation, affection factors may include abnormalities of blood flow in infralesion levels that influence bone cell differentiation, and hormonal changes that generate metabolic bone disorder

In the chronic phase of SL osteoporosis is balanced by increasing bone mineral density in regions of the body that are burdened by their own weight, like upper extremities and the spine, and even by greater demineralization of regions that are chronically without a load, such as the pelvis and lower extremities.

Parathormone (PTH) plays a significant role in the pathophysiology of osteoporosis induced by spinal cord lesion. In the acute phase of spinal cord lesion, and by the end of the first year after its occurence, PTH is almost inactive. (2) Return of its function appears from two to nine years after the occurrence of spinal lesion, but then reaches levels that are above the upper limit of physiological values, and then becomes a cause of osteoporosis in chronic phase of spinal lesion (3)

All methods used for the diagnosis of primary osteoporosis in the general population also apply to patients with SL. DEXA densitometry still remains the "gold" standard clinical methods of measuring bone density.

SL induced osteoporosis treatment is based on non-pharmacological and pharmacological treatment, provided that the non-pharmacological procedures are adapted to the patient's status. This is especially related to kinesitherapeutic procedures, primarily to passive standing.

The importance of prevention and treatment of osteoporosis caused by SL is reflected in preventing the occurrence of bone fractures, and the regulation of calcium metabolism and the consequent avoidance of visceral and ectopic muscular calcifications formation.

Objective

The aim of this study was to investigate and compare the PTH serum level in patients with chronic spinal cord lesion and subsequent osteoporosis before and after the therapy program, which consisted of alendronate and kinesitherapeutic procedures.

Patients and methods

A prospective, longitudinal, clinical, comparative study included 36 patients with chronic spinal cord lesion (24% or 67 men and 12 women, or 33%), who were diagnosed osteoporosis by DEXA densitometry of the lumbar spine and proximal femur. The average age of the patients was 41.7 ± 10.9 years and the median time of occurrence of spinal lesions was 12 years (IQR = 11 to 13).

Therapy program for osteoporosis consisted of weekly alendronate tablet of 70 mg, of daily calcium supplement of 500 mg, of vitamin D3 daily supplement of 400 IU, and of kinesithetapeutic procedures, which included passive standing and exercises, for the period of 18 months. Total daily period of standing was determined according to the T score of DEXA densitometry of proximal femur.

For patients with T score between -2 and -3 SD was recommended to stand for 2 hours daily , divided in 3-4 shorter period of time; for the patients with T score between -3 and -4 SD was recommended to stand for 1.5 hours daily, divided in 3-4 shorter period of time; for the patients with T score of -4 SD or below was recommended to stand for half an hour to an hour daily, divided into 3-4 shorter period of time.

Exercise program consisted of active exercises with and without resistance for the body parts above the SL, and of passive exercises for body

parts affected by the lesion. Therapy program was conducted at home. Controlling of program implementation has been carried every 6 months.

PTH serum levels were measured and compared before and after the therapy program.

After completion of therapy program patients were divided into 4 groups for statistical analysis:

(I) The first group consisted of patients who had performed the full therapy program (n=12), which means that they were regularly taking weekly alendronate tablet, performed standing within the prescribed time period and carried out the prescribed exercise program.

(II) The second group consisted of patients who did not perform passively standing (n = 5),

(III) The third group consisted of patients who were taking only alendronate tablets (n= 14),

(IV) The fourth group were patients who discontinued full therapy program implementation after 12 months (n=5).

The statistical tests were: Kolmogorov-Smirnov test, Independent Samples T-Test and Wilcoxon Signed Rank Test. A P value<0.05 was considered as significant.

Results

Before joining the therapeutic program 33% of patients had increased serum PTH level, average value of 63.16 pg/ml, and after performed program 20% of patients had increased level of PTH, average value of 49.74 pg /ml; (P<0.05). *Figure 1.*

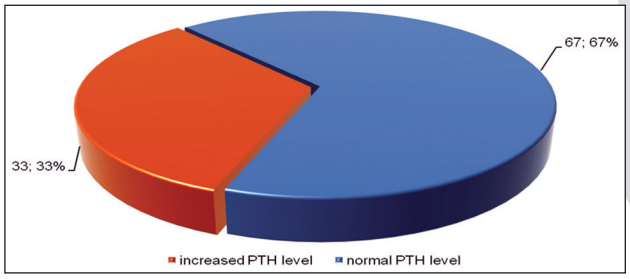

Figure 1. PTH serum level before therapeutic program

Increased PTH level correlated with worse DEXA densitometry finding of proximal femur. *Figure 2.*

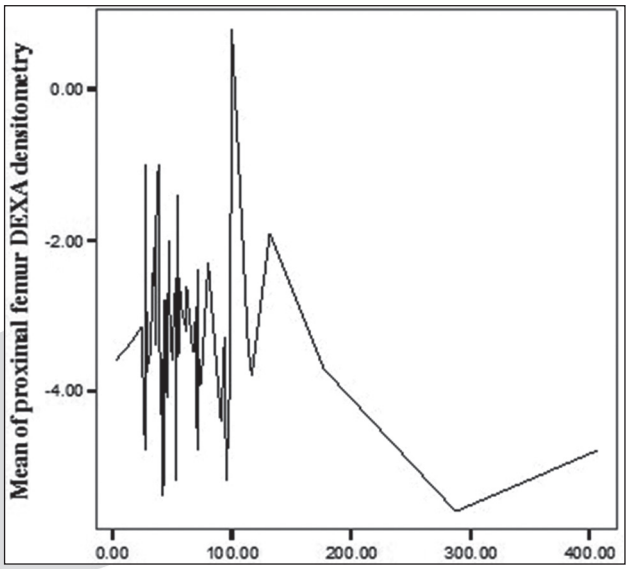

Figure 2. Correlation between proximal femur DEXA densitometry and PTH serum levels

There was statistically significant difference in median PTH level in males before (Me=51.8 pg / ml, IQR 40.1 to 68.9) and after the treatment (Me=39.7 pg/ml, IQR =27.0 to 61.5) (Z=-2.257, P=0.023), and in female before (Me=53.9 pg/ml, IQR=42.6 to 79.3) and after the treatment. (Me=41.0 pg/ml; IQR=28.4 do 61.3) (Z=-2.981; P<0.01). *Figure 3.*

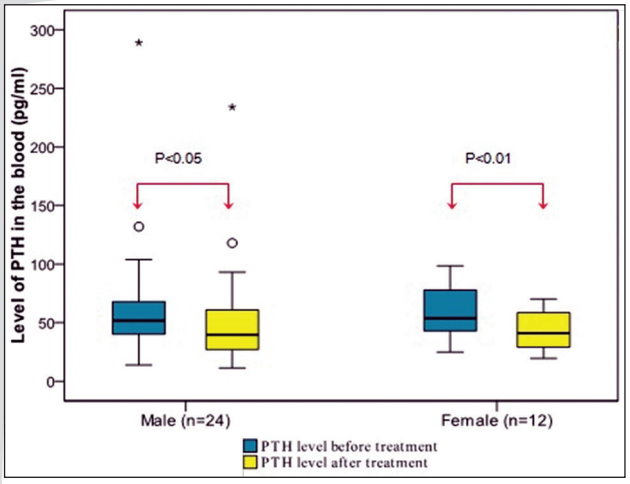

Figure 3. Level of PTH in the blood (pg/ml) before and after treatment according to gender (n=36)

In the age group of 20-44 years (*n=22*) median PTH level before treatment was 44.7 pg/ml (IQR=35.1 to 63.8), while after the treatment was 31.6 pg / ml (IQR=26.5 to 45.6), and the difference is highly statistically significant (Z=-3.685,

P<0.001). In the age group ≥ 45 years (*n=14*), median levels of serum PTH was 64.3 pg/ml (IQR=50.2 to 99.9) before the treatment, while after the treatment was 64.8 pg/ml (IQR=44.3 to 75.8), and the difference was not statistically significant (Z=-1.287, P=0.217). *Figure 4.*

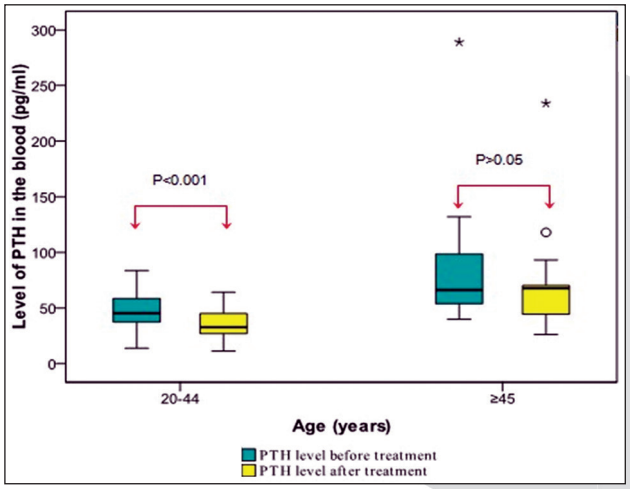

Figure 4. Level of PTH in the blood (pg/ml) before and after treatment according to age groups (n=36)

Significant decrease of serum PTH level had group I (t=3.37; P=0.04) and group IV (t=2.27; P=0.04). *Figure 5.*

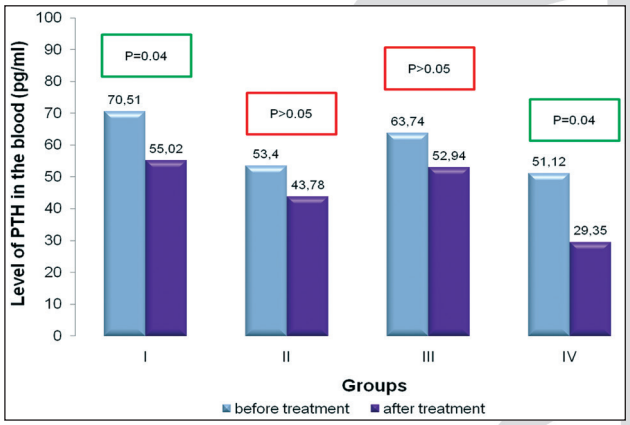

Figure 5. Level of serum PTH (pg/ml) before and after treatment according to groups (n=36) with different compliance to therapeutic program

Group I had the most significant improvement of T score DEXA densitometry of proximal femur. (for 0.59 SD). *Figure 6.*

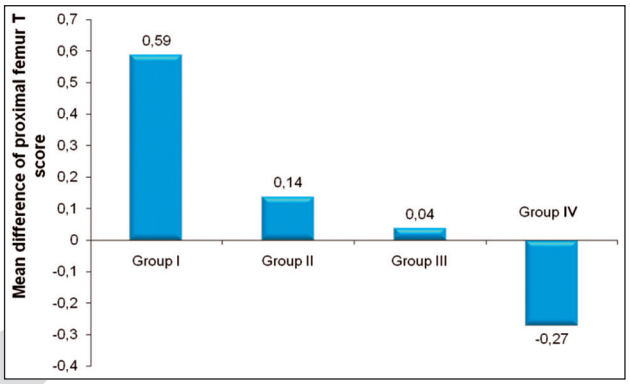

Figure 6. Mean difference of proximal femur T score between groups (n=36) with different compliance to therapeutic program

Discussion

In the chronic phase of spinal cord lesion leads there is metabolic disturbance of PTH - vitamin D axis. (4) The most recent study of Hummel and associates from the 2012th confirmed secondary hyperparathyroidism in patients with chronic spinal cord lesion, and its correlation with insufficient levels of serum 25-hydroxy vitamin D. (5)

The results of this study showed that 33% of patients had elevated level of serum PTH before joining the treatment program. There was a correlation of proximal femur DEXA densitometry lower findings and increased serum levels of PTH.

The same correlation was found in hemodialysis patients with osteoporosis. (6) Fast-acting effect of bisphosphonates leads to increased PTH serum levels due to decrease in the concentration of calcium in the blood. After that period calcium homeostasis is achieved, which leads to decline of serum PTH (7). It is important that the use of bisphosphonates is associated with a sufficient amount of vitamin D. (8)

Maintenance of normal bone axial load by passive standing (standing devices), or by use of various systems for walking, it was promoted as a therapy that reduces calcium loss and consequently retards associated SL osteoporosis for a long time. However, in patients with chronic SL and subsequent osteoporosis, application of just passively standing or walking with mechanical bracing showed no significant effect on bone mineral density (9).

Study of Güliz Gömül and associates from 2008th highlights that passive standing and active exercises for the upper extremity lead to a reduction of serum PTH in patients with SL (10).

In our study, following a therapy program in 60.6% of patients with elevated PTH serum levels there was decline to normal values of serum PTH. In relation to compliance, significantly reduce of PTH serum levels had the I group of patients who spent a full 18-month treatment program, and the IV group of patients who stopped implementation of full program after 12 months. However, significant improvement in proximal femur DEXA densitometry finding had only the I group.

It can be concluded that the implementation of full therapy program for a period of 12 months led to a reduction in serum PTH levels, whereas this period was not sufficient to improve the finding of proximal femur DEXA densitometry. In any case, passive standing contributed to the reduction of PTH serum level and improvement of proximal femur DEXA densitometry finding.

Conclusion

Eighteen months therapy program for osteoporosis consisting of weekly alendronate tablet of 70 mg, daily calcium supplement of 500 mg, daily vitamin D3 of 400 IU, and of kinesitherapeutic procedures, which included passive standing and exercise, has led to a lowering of PTH serum level in patients with chronic spinal cord lesion and induced osteoporosis, especially in the age group aged 22 to 44 years. More significant reduction had the group of patients who regularly performed passively standing within the prescribed therapy program.

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Therapeutic effect of arsenic trioxide intervention on the treatment of osteosarcoma pulmonary metastasis

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Abstract

Aim: To study the therapeutic effect of arsenic trioxide (As_2O_3) intervention on the treatment of osteosarcoma pulmonary metastasis.

Methods: 30 osteosarcoma pulmonary metastasis patients were involved. Femoral artery puncture and digital subtraction angiography (DSA) were conducted to locate the site and range of the tumor. Then two tips of the catheter were placed into the feeding artery of osteosarcoma and the bronchial artery respectively. They were administered with 30 mg As_2O_3 once/course that lasts one month. The therapeutic effects were evaluated after four treatment courses.

Results: The size of soft tissue mass of osteosarcoma in 10 patients was reduced by more than 50% and 15 cases by 10% -49%. The size of soft tissue mass of osteosarcoma in 3 patients was increased by more than 25% and that in 2 cases did not change whatsoever. The total effective rate was 83.3%. The size of pulmonary metastasis in 9 patients was dwindled by more than 50%, 17 cases by 10%-49% and that in 4 cases remained unchanged. The total markedly effective rate was 86.7%.

Conclusion: The interventional treatment with As_2O_3 for osteosarcoma pulmonary metastasis patients exhibits obvious short-term effect with scarce side effects.

Key words: As_2O_3, interventional treatment, osteosarcoma, pulmonary metastasis.

Introduction

Osteosarcoma is one of the common malignant tumors of bone with extremely high malignancy. Some patients have been complicated with pulmonary metastasis upon diagnosis, which does not allow surgeries [1-3]. Currently, common chemotherapeutic drugs are not sensitivity to osteosarcoma with high systemic chemotherapy dose and severe toxicity. Arsenic trioxide (As_2O_3), as a traditional Chinese medicine, has been verified to selectively induce osteosarcoma cells apoptosis, inhibit tumor angiogenesis and reduce the expression of vascular endothelial growth factor (VEGF) in tumor cells which is closely related to tumor metastasis [4-6]. Since 2002, interventional treatment with As_2O_3 has been applied in 30 cases of osteosarcoma pulmonary metastasis in our hospital, which was retrospectively analyzed as follows.

Materials and Methods

General information

30 patients were diagnosed as unresectable osteosarcoma pulmonary metastasis according to the results of X-ray plain film, CT or MRI and tumor feeding artery angiography, which included 25 males and 5 females aged between 13 and 55 years old with an average age of 34 years old. The lesions were located in the proximal tibia in 16 cases, 9 cases in the distal femur, 3 cases in the caput humeralis and 2 cases in the distal radius. The lesion diameters ranged between 4.5-8.5 cm with the average of 6.5 cm. All the 30 patients had soft tissue mass and pulmonary metastasis.

Methods

10 ml/unit arsenic trioxide injection containing 10 mg As_2O_3 (Harbin Yida Pharmaceutical Co., Ltd., National Medicine Permit No.: H19990191). 30 ml of arsenic trioxide injection was diluted to 100 ml with normal saline. Angiostar Plus large-sized C-arm X-ray machine was manufactured by SIEMENS (Germany). After successful femoral artery Seldinger puncture, the catheter tip was placed into osteosarcoma feeding artery for digital subtrac-

tion angiography (DSA) to further clarify the diagnosis, and then 70 ml of arsenic trioxide diluent was injected slowly. Then another catheter tip was placed into the bronchial artery for DSA to identify the number, sizes and scopes of metastases. Thereafter 30mg arsenic trioxide diluent were injected once with the treatment course of one month for four courses. X-ray plain film, CT or MRI were performed monthly to review the lesions.

Results

Short-term outcomes of osteosarcoma treatment

The size decreases of soft tissue mass of osteosarcoma after one to four courses are shown in Table 1 and Figure 1.

Short-term outcomes of pulmonary metastasis treatment

The size decreases of pulmonary metastasis after one to four courses are shown in Table 2 and Figure 2.

Side effects

The 30 patients suffered from preoperative tumor pain or discomfort which were alleviated after 1-6 days of administration. There were 4 cases of nausea after operation, 4 cases of renal function decline and 2 cases of liver function decline which were recovered after symptomatic treatment without significant leukocyte count decrease, bone marrow suppression, hair loss or apparent anorexia.

Follow-up

The 1-year survival rate was 85.7%.

Discussion

As_2O_3, commonly known as arsenic, was initially used in acute promyelocytic leukemia (APL) with identified effects [7-9]. Through in vitro culture of osteosarcoma strain MG-63 and human diploid cell strain MRC-5, Fang et al. [10, 11] found that As_2O_3 is obviously time-and dose-dependent in inhibiting the proliferation of osteosarcoma cells and diploid cells by inducing apoptosis. Xiao et al. [12] further confirmed that As_2O_3 can induce the up-regulation of p53 protein expression, so that the increased p53 protein combines with the IEX-1 gene promoter for the transcriptional inhibition of IEX-1 transcription, which thus down-regulates the expression of IEX-1 genes, suggesting that As_2O_3 can be used in the treatment of osteosarcoma. Although traditional syste-

Table 1. Short-term outcomes of osteosarcoma treatment Case (%)

Time	Case No.	Tumor volume decrease> 50%	Tumor volume decrease 10%-49%	Progress	Stable	Overall effective rate (%)
1st course	30	6 (20.0)	8 (26.7)	0	16 (53.3)	46.7
2nd course	30	7 (23.3)	10 (33.3)	0	13 (43.3)	56.7
3rd course	30	9 (30.0)	13 (43.3)	0	8 (26.7)	73.3
4th course	30	10 (33.3)	15 (50.0)	3 (10.0)	2 (6.67)	83.3

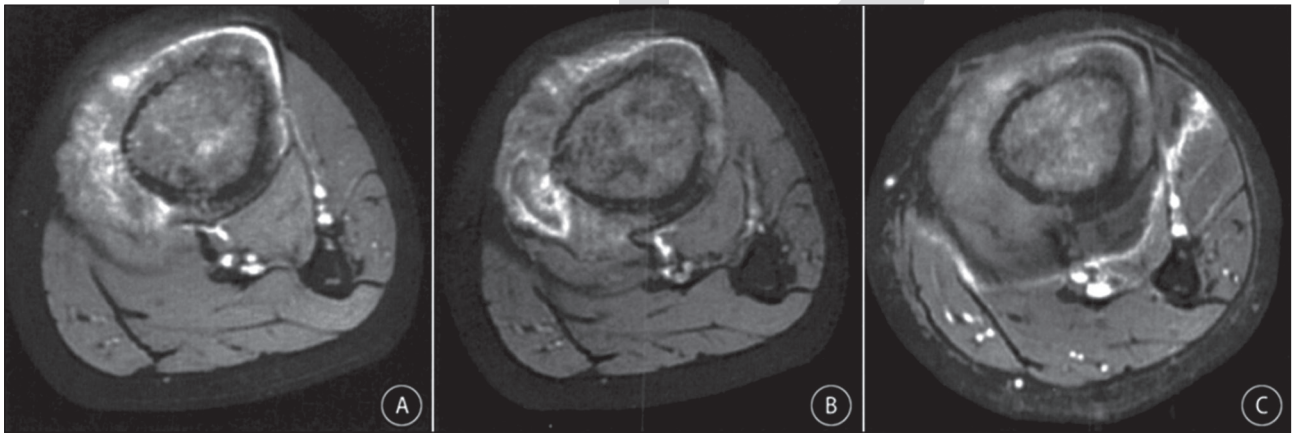

Figure 1. MRI before and after osteosarcoma treatment. A: axial-enhanced image before treatment; B: axial-enhanced image after the 2nd course; C: axial-enhanced image after the 4th course.

Table 2. Short-term outcomes of pulmonary metastasis treatment Case (%)

Time	Case No.	Tumor volume decrease> 50%	Tumor volume decrease 10%-49%	Progress	Stable	Overall effective rate (%)
1st course	30	5 (16.7)	10 (33.3)	0	15 (50.0)	50.0
2nd course	30	7 (23.3)	12 (40.0)	0	11 (36.7)	63.3
3rd course	30	8 (26.7)	13 (43.3)	0	9 (30.0)	70.0
4th course	30	9 (30.0)	17 (56.7)	0	4 (13.3)	86.7

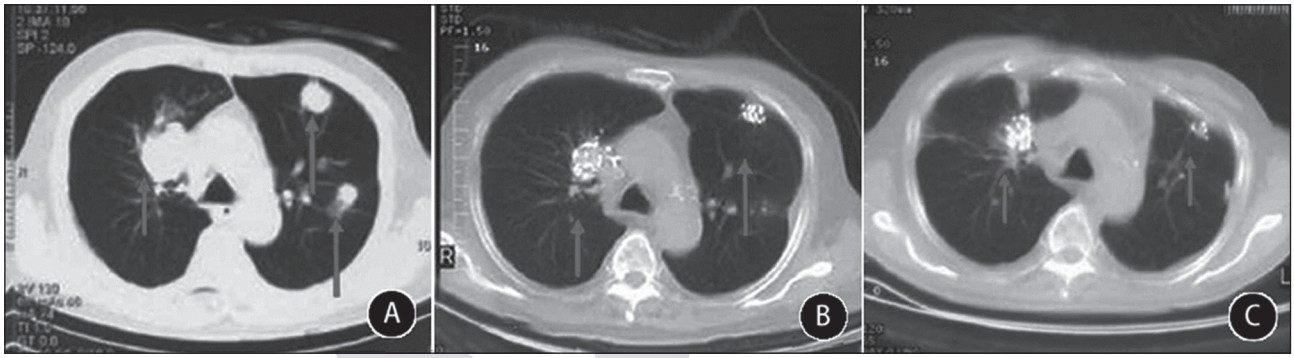

Figure 2. CT before and after osteosarcoma pulmonary metastasis treatment. A: bilateral pulmonary multiple metastasis before treatment; B:metastasis aggregated and tumor size decreased after the 2nd course; C: tumor size was further reduced after the 4th course.

mic chemotherapy has tolerable curative effect, it has many side effects [13, 14]. Tumor feeding arterial chemotherapy is one of the effective methods as a result of reducing systemic side effects due to a significant increase of drug concentration in the tumor [15, 16]. We formulated a new chemotherapy regimen for osteosarcoma pulmonary metastasis by tumor arterial perfusion: 30 ml of As_2O_3 for P times (osteosarcoma with 20 ml of As_2O_3 for P times + pulmonary metastases with 10 ml of As_2O_3 for P times), once P treatment courses, with a treatment course of P months. The therapeutic effects were evaluated after four courses. After four courses of As_2O_3 treatment, this study shows that: (1) As_2O_3 is evidently time-dependent on the osteosarcoma treatment, and can effectively mitigate the pain of tumor region and improve the quality of life. Most of the patients experienced alleviated pain symptoms of tumor after 1-6 days of administration, inferring that it is related to the decreased size of soft tissue mass of osteosarcoma. (2) One-time intubation and multi-site tumor arterial perfusion chemotherapy lead to slight systemic side effects. It has previously been reported that the side effects are mild and reversible in the leukemia treatment with As_2O_3 [17, 18]. In this study, only 3 cases of nausea were observed without decreased leukocyte count, fatigue, hair loss or significant side effects on the liver and kidney. (3) As_2O_3 has remarkable short-term effects. The overall effective rate was 84.2% and the 1-year survival rate was 81.2%. We believed that As_2O_3 is a potent chemotherapy drug for the osteosarcoma pulmonary metastasis patients with considerable therapeutic value and low toxicity.

It is worth noting that relevant clinical studies are being conducted on whether the dose for the treatment herein can be increased, whether the increased dose can enhance the curative effect, and how the treatment functions with other chemotherapy drugs. The 3-year survival rate is now being followed up. Some osteosarcoma patients with pulmonary metastasis underwent arthroplasty P prosthesis implantation after the size of soft tissue mass was decreased by tumor arterial As_2O_3 perfusion chemotherapy. The short-term and long-term effects are being followed up. These patients were not included in this study.

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Etiopathogenesis and surgical physiology of inguinal hernias

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Abstract

Introduction: Hernia is one of the most frequent surgical diseases. Hernia represents a protrusion of preperitoneal adipose tissue and peritoneum either with visceral organs or without them, through congenital or acquired weakened point in the abdominal wall or in the abdomen. In every hernia there is a defect of musculoaponeurotic and fascial continuity with an opening in the wall of the abdomen or intreperitoneally in dilated pockets of the peritoneum.

Objective of the paper is to remind ourselves of the basic knowledge about the nature of this disease, to point to the contemporary views in the area of etiopathogenesis, and to establish the incidence of surgeries of inguinal hernias in relation to the total number of those operated on in the Medical System Belgrade in the period from 01/01/2003 to 31/12/2012, and to present the types of surgical techniques that are applied in surgical management of inguinal hernias.

Materials and methods: The study is of retrospective-prospective character and covers 1460 patients operated on for inguinal hernia in the Medical System Belgrade. The study was conducted as prospective in the period from 01/01/2008 to 31/12/2012 and, as retrospective in the period from 01/01/2003 to 31/12/2007. In that period, in the Medical System Belgrade, 4380 patients were surgically treated. The study covers patients operated on for inguinal hernia, either direct or indirect, while the data on femoral and incisional ones were not processed. The data from case histories were used and, in patients who were operated on in the prospective part of the study, in addition to case histories, questionnaires were used, which were filled in by patients at admission. Patients were classified according to the localization of hernias and according to the type of surgical technique that was applied on inguinal hernias.

Results: In relation to the total number of those operated on, 4380, in the Medical System Belgrade, those operated on for hernias were 1560 (35.6%), and there were 2820 (64.4%) other surgeries. When localization of hernias is in question, there were 1420 (91%) surgical interventions due to inguinal hernias, 38 (2.4%) due to femoral ones, 62 (4%) due to umbilical ones, 21 (1.35%) due to epigastric ones, and 19 (1.25%) due to incisional ones. In surgical management of inguinal hernias, in the period of 2003-2005, 'tension' and tension-free surgical techniques were almost equally applied. 'Tension' surgical techniques constituted 46%, specifically most frequently Halsted and Bassini ones, and tension-free accounted for 54%, specifically most frequently Lichtenstein technique. In the period from 2006-2012, tension-free surgical techniques were most frequently applied, specifically in 95% of the cases.

Conclusions: Hernia is one of the commonest surgical diseases. Modern views of etiopathogenesis of hernias indicate that anatomic-structural disorders are determined by biological changes on molecular level. Incidence of interventions on hernias is very high, 35.6%, in relation to the total number of those operated on in the Medical System Belgrade. According to the localization, the most frequent are inguinal hernias, with 91%. The commonest surgical techniques that are applied in management of inguinal hernias in the Medical System Belgrade are tension-free ones, specifically of Lichtenstein type.

Key words: Hernia, inguinal hernia, surgical technique.

Introduction

When nothing was yet known about the anatomical relationships between abdomen and scrotum, Celsus, way back in the first century B.C., described the contents of hernia and reported the

difference between hernia and hydrocele (1). In the second century A.D., Galen concluded that hernias occur by cleavage of the peritoneum, and then on that base, cleavage of fascia and muscular weakness in that region (2) occur as well. In the course of history, some authors connected hernia with the presence of testicles in that region. For surgery to be developed as well as the entire science, one of the important determinants was the development of anatomy. In the 14th and the 15th centuries, in Bologna, Venice, Vienna, Padua, and Paris, anatomical dissection was introduced and that resulted in greater progress of anatomy (1). In the 18th century, Percival Pot described congenital hernia (2) for the first time. A. Cooper was particularly engaged in the study of anatomy of inguinal region, and he, in 1807, issued the paper: Anatomy and Surgical Treatment of Inguinal Hernias. In his paper, particularly winning high marks is the description of the internal inguinal ring, inguinal canal, and funiculus spermaticus, observation of the direction and flow of the spermatic cord, and the description of the transversal fascia and sheath of femoral blood vessels. He also described the ligament, which is called after him: lig. Pectineale Cuperi. He then formulated the theory that hernia is the consequence of insufficiency of the internal, and not the external opening of the inguinal canal, and he also gave a detailed description of the posterior wall of the inguinal canal and noted that its strength depends on the transversal muscle and transversal fascia (3).

Papers of a more decent date demonstrate the real importance of transversal fascia, and they originate from Anson, Mcvey, Nyhus, and Condon. They describe the real significance of iliopubic tract as the derivative of transversal fascia (1). Mcvey, in 1942, underlined in his paper that Poupart's ligament is not the area of origin, neither insertion of transverse abdominal muscle, nor of internal oblique one. In his paper, he particularly stressed the difference between aponeurosis and fascia, where he said that aponeurosis is a fibrous extension of myofiber, and the tendon is a thin layer of stroma that covers muscles and aponeuroses (3). In England, Steel ligated hernia on the level of the external inguinal canal, and Kohler applied sutures on aponeurosis of the external abdominal muscle. Championniere, in 1881, opened fascia of the external oblique muscle and left the inguinal canal open, and performed doubling of fascia externally (4). All those attempts resulted in the mortality rate of around 7%, relapses of 40% within up to one year, and 100% in the course of 4 years.

Disappointed by the results, surgeons generally performed excisions of hernial sacs and left wounds to heal up per secundam, including carrying trusses postoperatively. Then, Eduardo Bassini appeared who with his monumental works stopped failures of the up to then interventions (5).

Hernia is one of the most frequent surgical diseases. Hernia represents protrusion of preperitoneal adipose tissue and peritoneum either with visceral organs or without them, through congenital or acquired weakened point in the abdominal wall or in the abdominal cavity. The abdominal wall is a laminar musculoaponeurotic functional whole which, together with the vertebral column, closes the abdominal cavity. The abdominal wall is divided into the anterior frontal and posterior, and the borderline is the vertical line drawn through the tip of XII rib. Borderlines of anterolateral abdominal wall are: upward, costal arches and xiphoid extension, down, crista iliac, pubic bone, and symphysis, medially central line (linea alba), and laterally, the vertical line passing through the tip of XII rib (4). Anterolateral wall is formed of soft parts distributed in seven layers (skin, subdermis, fascia investiens abdominis, musculoaponeurotic layer, fascia endoabdominalis seu transversalis, fascia extraperitonealis, and peritoneum).

The front abdominal wall has spots in which it is less resistant. Hernias appear on such areas. In view of the fact that resistance of the abdominal wall mainly depends on its musculoaponeurotic layer, weak areas are located at places where muscles are spaced apart from one another. Moreover, weak points are located in the region of the openings on the musculoaponeurotic layer, which serve for the passage of blood vessels and nerves. Weak spots are the inguinal canal and openings on the white line.

Inguinal region is a part of the anterolateral abdominal wall delimited upward, by bispinal line, downward and laterally, by inguinal ligament, and inside, by the external edge of m.rectus abdominis. Inguinal region contains the inguinal canal. It is located in the front abdominal wall above the internal half of the inguinal ligament. It is around 4 cm long, and it is projected on the line of symmetry of

the angle formed by the external edges of m.rectus abdominis and plica inguinalis. The spermatic cord and round uterine ligament in women pass through this canal. On the inguinal canal we differentiate the anterior and posterior, upper and lower walls and a surface and a deep inguinal opening. The front wall is formed by aponeurosis of m.obliquus externus abdominis. The posterior wall is formed only by a thin fascia transversalis coated on the posterior side by parietal peritoneum (1,2,3,4).

In every hernia there is a defect of musculoaponeurotic and fascial continuity with an opening in the wall of the abdomen or intreperitoneally in the dilated pockets of the peritoneum (6). Hernias are classified as congenital (hernia congenita) and acquired (hernia acquisita). If a hernia is in the abdominal wall and visible, then it is external and, if it is located in doublings of the peritoneum or between the abdominal cavity and chest barrel, it is an internal hernia. Reducible hernia is the one the content of which can be pushed back into the abdominal cavity, and irreducible is the one the content of which cannot be pushed back. Hernia acreta is the one in which the contents of hernia have adhered to the hernial sac.

According to their localization, hernias can be inguinal, femoral, umbilical, and hernias of the white line. Incisional hernias develop on the base of the scar from the previous surgery, and infections, obesity, poor drainage, and other postoperative complications contribute to their development. Recurrent or relapsing hernias are those that reappear after a surgery. Double and triple hernias are those developing on two or more openings and may have the appearance of saddle-bags or trousers.

Hernias are determined according to their localization, according to the condition, size, cause, and according to their contents. Every hernia has a hernia gate, hernial sac, contents of hernia, and hernia coverings. Hernia gate is the weakened spot in the abdominal wall through which a hernia protrudes from the abdominal wall through weak points: inguinal region, openings on the white line, and femoral region. In internal hernias, gate is located in the diaphragm, recesus ileocekalis, and formen epiploicum.

In an incipient hernia (hernia incipiens), hernia gate is only in weakened, posterior layers of the abdominal wall and, in a complete hernia (hernia completa) there may be a defect of all the layers of the abdominal wall except for the skin and peritoneum. Hernial sac is the protrusion of the parietal peritoneum through hernia gate. It may be congenital and acquired. It may happen that, along with peritoneum, some abdominal organ also slides through the hernia gate, which is not covered by parietal peritoneum (cecum, colon, urinary bladder) and which together with visceral peritoneum constitutes a part of the wall of the hernial sac. These organs are partially retroperitoneal, and their sliding through the hernia opening is compared with movements of glaciers. Such hernias are called sliding hernias. On a hernial sac, we differentiate the mouth, neck, body, and fundus. Hernial sac may have one or more cavities. Hernia coverings are located on the external side of the body and fundus of hernial sac, and may consist of all the layers of the abdominal wall: preperitoneal adipose tissue, musculoaponeurotic structures, fascia, panniculus adiposus, and skin. Most frequently sheaths are formed of preperitoneal adipose tissue that many wrongly call prehernial lipoma. Content of a hernia may include all the abdominal organs that pass through the hernia opening. Most often those are small intestines and omentum, and may also be the urinary bladder, colon, ovary, cecum, etc. In particularly large hernias, all the organs of the abdominal cavity can be found in the hernial sac and, therefore, their reposition is very difficult, because there is no longer room for them in the stomach.

In the clinical picture of hernias, subjective symptoms are various. They generally depend on the localization and condition of the hernia, as well as on whether the hernia is getting complicated or not. Difficulties occur because of the dysfunction of organs that are contained in hernia and of the dysfunction of the abdominal wall the strength of which is weakened due to existence of hernia. The possibility of exertion of the abdominal wall is reduced, because difficulties intensify during exertion (7). Sometimes a patient does not have any difficulties and does not know that he/she has got a hernia. Sometimes pain also appears, specifically during a powerful muscular effort, and it is not proportional to the size of the hernia. However, smaller hernias cause more intensive difficulties, because a smaller hernia gate produces stronger constriction and causes greater difficulties (6,7,8).

Case history and clinical examination are, in a majority of cases, sufficient to make a diagnosis of hernia. During the examination, it is very important that the patient is without clothes, that the region of the abdomen and genitalia is clearly visible, and to ask the patient to show where he/she has identified difficulties. (6,8,9). In indirect hernias, it is necessary to palpate the external inguinal ring and then to proceed with the entering procedure, i.e. to pull the finger from the external opening towards the internal one of the inguinal canal. In the course of that procedure, it is important to establish weakness of the posterior wall of the inguinal canal. If, during entering, we notice that the finger freely enters towards the abdominal cavity, the diagnosis is clear. When there is no hernia, not even the finger cushion can enter in the external opening (9). Sometimes hernias are so large that taxis should be performed, and then further testing if we suspect that a combined hernia is in question. In direct hernias we apply digital pressure on Hesselbach's triangle and then we can easily palpate Cooper's ligament, which is not the case in indirect hernias. Direct hernias never protrude into scrotum, i.e. never cross the penis base (6).

The assessment whether direct or indirect hernia is in question can be made in relation to the pulsation of the inferior epigastric artery. If, during entering, a hernia is medially from the pulsations of this artery, a direct hernia is in question and, if the same pulsates laterally, an indirect one is in question (6). If we talk about femoral hernias, their characteristic is that they pass below the line that interconnects iliac spine and tuberculum pubicum (10).

In a patient with ileus, it is necessary to devote attention and to see whether there is a visible hernia or a smaller hernia or an interstitial or internal hernia present, which may be the cause of ileus. If we have an incarcerated hernia, tumefact in inguinum is noticed during examination, which is not reduced and, anamnestically, we get the datum on the hernia, which is now irreducible, and the patient has nausea, sickness, and vomiting or complete symptoms of ileus (10).

Differentially diagnostically, lipomas can be considered, which are frequent in this region, but have a constant size and do not have the hernia neck. We can confuse swollen lymph nodes with a femoral hernia. A hydrocele does not change shape and size either under strain or pressure, it is painless and phototransparent. Venous varicosities in fossa ovalis can be confused with a femoral hernia, but they are reduced when pressure is applied, and promptly thereafter they bounce back. There is blood vibration in them. A varicocele is usually on the left side in a testicle and it does not cause any major diagnostic difficulties to an experienced surgeon (9).

There are numerous theories of the etiopathogenesis of hernias. Early discovery of procesus vaginalis gave rise to the formulation of the congenital theory of development of hernias. This theory deems that the cause of hernias is persistent procesus vaginalis and, as treatment, high legation of the hernia sac (1,3) is recommended. Keith accepted the importance of procesus vaginalis in certain cases, but he observed fascial structures as live tissues with a dynamics, and explained the increased incidence of hernias in older people as the consequence of pathological changes on stroma (2). Harrison claimed that changes on transversal fascia are the cause of weakness of the inguinal region and inability to resist increased intraabdominal pressure (2,3).

For onset of a hernia it is necessary that a defect exists in musculoaponeurotic and fascial structures of the abdominal wall at the spot where the hernia gate is created. It is necessary to have a certain predisposition and a hereditary component, but also important are the age, gender, obesity, and other factors (10). One of the most important factors is increased intrabdominal pressure, which occurs due to the strain of the abdominal wall. Increased pressure may occur acutely due to sudden powerful muscular efforts or lifting of heavy loads or may onset chronically due to pregnancy, ascites, constipation, and similar conditions.

Etiopathogenesis of formation of the hernia gate is an important moment in development of hernia although creation of hernial sac also plays an important role, albeit there is no full explanation of the etiology and pathogeneses of hernias as yet. Worth mentioning is Cloquet's so-called lipoma theory of development of hernia. It says that, in the beginning of development of a hernia, preperitoneal adipose tissue protrudes into the weakened spot in the abdominal wall and that it drags peritoneum along, which bulges at that spot in the form of a diverticulum. Any abdominal organ

may enter in the diverticulum. This theory is now acquiring significance although it was considered obsolete for some time (1).

Modern views have evolved from the above ones towards biological factors as primary factors of etiopathogenesis of herniation. Abnormalities that are ultimately manifested as anatomical-structural disorders are primarily determined by biological changes on molecular level. The issue of vaginal procesus takes on a whole new meaning. The process of obliteration of vaginal procesus is caused by changes in the formation of fibrous tissue. The entire vaginal procesus between the two openings is coated by myofibroblasts, i.e. cells that contain contractile proteins. This creates the possibility of higher production of stroma, which obliterates peritoneum at the point of the beginning of vaginal procesus on the level of the internal ring. Subsequent accumulation of collagen along the canal results in its complete obliteration. In view of the fact that 50% of children have persistent vaginal procesus at birth and, up to puberty, a half of them are without complete obliteration, while only a minor number develop hernia, it can be concluded that, in children, persistent vaginal procesus may be predominant, but not the exclusive cause of hernia (1,3).

Similar studies demonstrate that one of the factors of development of hernia may be primarily a metabolic disorder, i.e. loss of quality of fascia and aponeurosis of the posterior wall of the inguinal canal (2). Essentially, the synergy of a number of factors gives rise to the development of hernia in the predominant number of cases.

Analyzing causes of relapses, Peacock found that the cause of their development is not poorly ligated hernial sac, but further progression of the pathological process on the level of the internal ring and, therefore, the attempt to repair it in such cases is the attempt to surgically resolve a metabolic defect (2). Finally, we can conclude that there is no definitive etiological theory of herniation as a pathological process, but so-far research works provide to a surgeon a high degree of certainty in classification and selection of the surgical technique.

Objective

Several objectives were set in this paper. Objectives of this paper are to:

- Remind ourselves of the basic knowledge about the nature and diagnostics of hernias
- Point to contemporary views in the area of etiopatogenesis of hernias
- Determine incidence of surgeries of inguinal hernias in relation to the total number of those operated on in the Medical System Belgrade in the period from 01/01/2003 to 31/12/2012.
- Present the types of surgical techniques that are applied in surgical management of inguinal hernias.

Materials and methods

The study is of retrospective-prospective character, covering 1420 patients operated on for inguinal hernia in the Medical System Belgrade. The study was conducted as prospective in the period from 01/01/2008 to 31/12/2012, and as retrospective in the period from 01/01/2003 to 31/12/2007.

In that period, in the Medical System Belgrade, 4380 patients were surgically treated. The study covered patients operated on for inguinal hernia either direct or indirect, while the data on femoral and incisional ones were not processed.

The data were used from case histories and, in patients who were operated on in the prospective part of the study, in addition to case histories, questionnaires were used, which were filled in by the patients at admission.

Patients were classified according to the localization of hernias, according to the type of inguinal hernia, and according to the type of surgical technique that was applied on inguinal hernias. The data were processed applying modern methods of descriptive and analytical statistics. The results are shown in tabular form and graphically.

The questionnaire, which was drawn up for the purpose of the prospective part of the study:

Name and family name.....
Place and year of birth.....
Have you had any diseases and which ones?.....
.....
Do you suffer from diabetes (if yes, since when)?.....
Do you suffer from asthma (if yes, since when)?.....
Have you undergone any surgeries (which ones and when)?.....
Since when have you had hernia?.....
How did it develop (suddenly or gradually)?.....
Has it been jammed and how many times?.....
Can the hernia you have got be reduced or it is always present without the possibility of reduction?
.....
Is there anyone with a hernia in the family?.....
Have you been operated on for hernia before?.....
When were you operated on and were you operated on on the same side and have you hernia now?
.....
Who was the surgeon that operated on you?.....
Did you have an infection or suggillation in the wound then?.....
On what postoperative day did you leave the hospital?.....

Results

Out of the total number of surgeries, 4380, surgeries on hernias were performed on 1560 patients, which accounts for 35.6% of the cases. Applying the method of chi-squared test for pair-matched samples, we tested to see whether incidence of hernia is significant in the total number of those operated on.

Table 1. Number of those operated on in the 2003-2012 period

Type of surgery	Incidence	%
Hernia surgeries	1560	35.6
Other surgeries	2820	64.4
Total surgeries	4380	100

The value of chi-squared is 4.567; $p < 0.05$. The obtained difference is statistically significant.

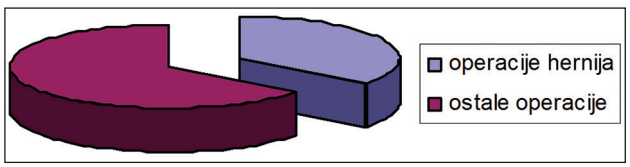

Diagram 1. Number of those operated on in the 2003-2012 period

As to the localization, the most frequent type of hernias is inguinal. The difference is highly statistically significant, chi-squared is 344.45; $p< 0.01$. This difference originates from a pronounced predominance of inguinal hernias, which appear in as many as 91% of all the cases of hernia, and all the other types account for 9% of hernias.

Table 2. Number of those operated on for hernias by the localization of hernias in the 2003-2012 period

Localization of hernias	Number of those operated on	%
Inguinal	1420	91.0
Femoral	38	2.40
Umbilical	62	4.00
Epigastric	21	1.35
Incisional	19	1.25
Total hernias	1560	100

In the 2003-2005 period, 452 patients were those operated on and, in surgical management of inguinal hernias, 'tension' and tension-free techniques were almost equally applied. In that period, 'tension' surgical techniques constituted 46%, specifically most frequently Halsted and Bassini

ones, and tension-free accounted for 54%, specifically most frequently Lichtenstein.

Table 3.1. Share of surgical techniques in inguinal hernias in the 2003-2005 period

Type of surgical technique	Number of those operated on	%
'Tension' techniques	208	46
Tension-free techniques	244	54
Total surgeries	452	100

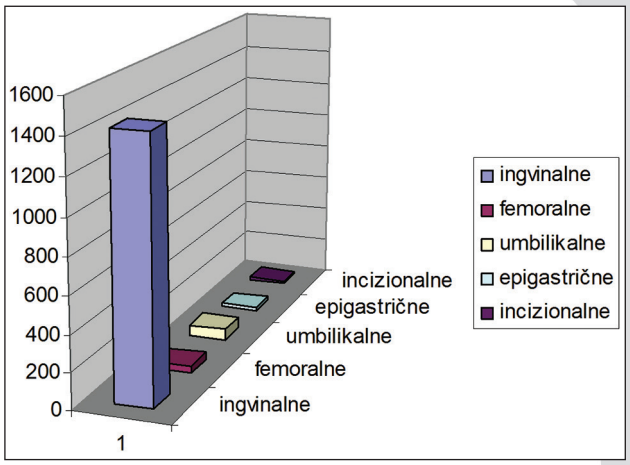

Diagram 2. Number of those operated on for hernias by the localization of hernias in the 2003-2012 period

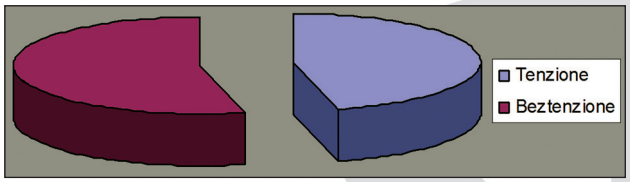

Diagram 3.1. Share of surgical techniques in inguinal hernias in the 2003-2005 period

In the 2006-2012 period, 968 patients were operated on and, in relation to type of surgical technique, statistically highly significant are surgeries of tension-free type. They constitute 95% of surgical techniques involving inguinal hernias (ANOVA for proportions F=8.786; p<0.01). Within this group, Lichtenstein surgical technique (F=4.245; p<0.05) statistically significantly stands out.

Table 3.2. Share of surgical techniques in inguinal hernias in the 2006-20012 period

Type of surgical technique	Number of those operated on	%
Tension-free techniques	920	95
'Tension' techniques	48	5
Total surgeries	968	100

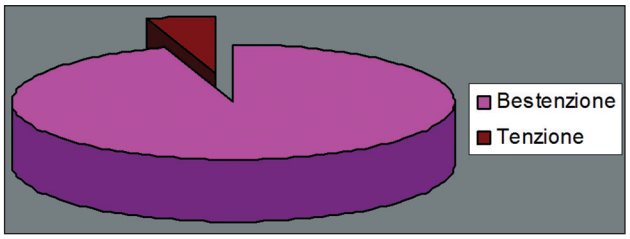

Diagram 3.2. Share of surgical techniques in inguinal hernias in the 2006-2012 period

Discussion

Out of the total number of those operated on in the Medical System Belgrade, the number of those operated on for hernias was statistically significant, 35.6%, which corresponds to the data from literature evidencing that hernias are the commonest surgical operations (6). As to the localization, the most frequent type of hernias is inguinal. The difference is highly statistically significant, chi-squared is 344.45; p< 0.01 in relation to other types of hernia, which corresponds to the data from literature. This difference originates from a pronounced predominance of inguinal hernias, which appear in as many as 91% of all the hernia cases, and all the other types account for 9% of hernias (11).

In the 2003-2005 period, in surgical management of inguinal hernias, tension-free and 'tension' surgical techniques were almost equally applied. In that period, tension-free surgical techniques constituted 54% including Lichtenstein type, and 'tension' ones accounted for 46%, specifically of Halsted and Bassini types. In the 2006-2012 period, as to the surgical technique, statistically significant are the surgeries of tension-free type. They constitute 95% of surgical techniques on inguinal hernias (ANOVA for proportions F= 8.786; p < 0.01). In this group of surgical techniques, Lichtenstein type (F = 4.245; p< 0.05) stands out as a statistically significant one. Based on such incidence, we may conclude that, in surgeries of inguinal hernias in the Medical System Belgrade, tension-free surgical technique is applied in the majority of cases, specifically of Lichenstein type, which corresponds to the data from literature (12).

It is also necessary to point to the fact that the selection of a surgical technique to a large extent depends on the age of the surgeon. In the Medical System Belgrade, a surgeon of older age (65 years

old) has applied 'tension' surgical techniques of Halsted and Bassini types on all of his/her patients, a somewhat younger surgeon (50 years old) has applied 'tension' surgical techniques in 28% of the cases, and tension-free in 72% of the cases, while a young surgeon (35 years old) has applied tension-free surgical technique on all the patients, specifically of Lichtenstein type.

Conclusions

Hernia is one of the most frequent surgical diseases.

Modern views of etiopathogenesis of hernias indicate that anatomical-structural disorders are determined by biological changes on molecular level.

Incidence of interventions on hernias is very high, 35.6%, in relation to the total number of those operated on in the Medical System Belgrade.

According to the localization of hernias, the most frequent are inguinal hernias, with 91%.

The most frequent surgical techniques that are applied in surgical management of inguinal hernias in the Medical System Belgrade are tension-free, specifically of Lichtenstein type.

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Post-infarction heart failure in Diabetes mellitus

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Abstract

Background: At work on internal Department we noticed frequent occurrence of heart failure in postinfarctial flow in diabetics and that with severly clinical course.

Materials and Methods: Retrospective study were tested total of 276 hospitalized patients in the RMC " Dr.Safet Mujić " Mostar with and without diabetes with signs post-infraction heart failure and age of 40-60 years and over 60 years of age. Patients were divided into two groups: A group of 134 diabetic patients and group B of 142 patients without diabetes. Here were investigated Echocardiographic values of the ejection fraction, clinical symptoms according to the NYHA classification, duration of hospitaliyation and influence on the clinical course of post-infarction heart failure.

Results: Research has show that severe clinical course of post-infarction heart failure comparated with non-diabetic pacients of the same age. According to the NYHA classification diabetics are more numerous with more severe degrees of heart failure compared with nondiabetic patients, and there is a statistically significant difference between the two groups, with a p value of 0.004 tj.p <0.05. The average duration of hospitalization stay was longer in diabetics compared to nondiabetics and the t test that is p <0.05. Values of ejection fraction below 40% is more numerous in diabetics than nondiabetics.

Conclusion: Diabetes mellitus type 2 is a significant independent factor in the development of myocardial infarction and severe post-infarction clinical course and the of heart failure.

Key words: Diabetes mellitus, heart failure, myocard infarct.

Introduction

Diabetes mellitus type 2 is a severe progressive disease, characterized by two fundamental defect of: insulin resistance and β-cell dysfunction (1,2). Progressive nature of the disease and lack of glycemic control as a consequence of the weakening of the function of pancreatic β cells with the upcoming reduction in insulin secretion (3). Hyperinsulinemia is not sufficient to overcome the increased concentrations of glucose, results in hyperglycemia runs glukotoksic, which adversely affects the pancreatic β cells, and in addition there is a genetic defect in the conditioned mass and function of β cells, which eventually leads to the occurrence of type 2 diabetes. (4) Recent studies have found that people with normal weight and the person appears to insulin resistence and type 2 diabetes mellitus.(5,6)

Increasingly, the literature mentions Maturity-Onset Diabetes (MODY),which usually occurs before twenty-five years of age often in childhood and adolescence. The primary disturbance in the function of pancreatic β cells, and it is a heterogeneous group of disorders with non-ketotic diabetes mellitus, which is inherited autosomal dominant. (7)

Many studies have shown a link between diabetes and obesity and the degree of excess body weight.(8,9,10). In the course of diabetes, are begining severe changes in the cardiovascular system in the forum of micro-and macrovascular complications especially if the illnes last longer than 10-15 years,reardless of the age of the patient.Aterosclerotic heart disease is more common in dibetics than in the general population. Coronary artery disease and heart failure are intimately associated with diabetes and obesity. (11,12)

Myocardial infarction in 2-3 times more common in diabetics caused by disturbance of autonomic function and ateroosclerotic processes. Very serious complications in diabetic patients

is diabetic cardiomyopathy, wich usually leads to heart failure, wich is a weakness of the inabiliti of the heart to the normal volumes and filling presures ejected cardiac output that meets the metabolic needs of the body.

Target of work

It's important to determine whether there is influence and connection type 2 diabetes mellitus with the development of more severe clinical course of post-infarction heart failure. Followuing two groups of patients of the same age, wich are diabetics and nondiabetic pacients with postinfarction heart failure show severe clinical course of patients with diabetes and the early detection and adequate therapeutic approach that early with insulin teraphy treat diabetes and that way prevent aterosclerotic changes on the entire cardiovascular system.

Patients and Methods

Subject diabetics with postinfarction heart failure at the age of 40-60 years and over 60 years of age who hospitalized at Department of RMC"Dr.Safet Mujić" Mostar, for period of 4 years were monitared and tested. Hospitalized diabetics 134 of thwem with electrocardiografic signs of infarction anterior, anterolateral, inferior localisation and with development signs of heart failure according to the NYHA classification wich had values of the ejection fraction under 50% and over 50%. It was folowed lasting of hospitalization under 7 days, foom 8-14 days, and over 15 days.Control group was from 142 hospitalized patients nondiabetics mellitus and it was formed on similar way. All of patiens werw made basic laboratory elaboration, clinical examination because of NYHA clasification, EKG snapshot, and Ehokardiografic values ejekcion fraction.

In retrospective study wich lasted 4 years(since march 2009 year until march 2013 year werw examined total 276 patients (man and womans in age from 40 to 60 years and over 60 years of age in two groups: A group (134 diabetics with postinfarction heart falilure) and B group (142 nondiabetic mellitus patients with postinfarction heart failure). Examinnation is devised on following and collecting from piece of information from historys of disease hospitalized patients in RMC "Dr. Safet Mujić" Mostar and that diabetics and nondiabetics with postinfarction heart failure in last 4 years. There were examined laboratory charakteristics of values glucosion in blood, EKG snapshot of localisation infarction, Echocardiographic values of the ejection fraction according clinical symptoms of the disease to the NYHA classification, diration of hospitalization and influence on the clinical course of post-infarction heart failure. By clinical examine is followed atetndance of dyspnea in biger efforts in usual efforts in minor efforts also as attendance of dyspnea in rest.

(Heart failure according to NYHA classification). By echocardiography are demonstrated akinesia, hypokinesia, dyskinesia, which are the consequences of myocardial infarction. All patients was performed echocardiography and information on the value of ejection fraction were analyzed because Echocardiography in real-time technique with B and M screen and the ejection fraction is the most practical method for determing heart failure. (13)

Result

Using statistical anallysis Social Sciences for Windows 17 (SPSS Inc., Chicago, IL, USA). Using Pearson linear correlation and Spearman rank correlation was shown the existence of a strong link beetween „diabetes mellitus typ 2 and heart failure" (Table 1) and „Diabetes mellitus typ 2 and duration of hospitalization„ (Table 3). After statistical analysis of the data showed that diabetics are more numerous and with NYHA III and NYHA IV (Figure 1) or more severe degrees of heart failure compared with nondiabetic patients who are more numerous with with NYHA I and NYHA II (Figure 2) that means mildnes degree of heart failure and there is a statistically significant difference between the two groups, with a p value of 0.004 $p < 0.05$. Average duration of hospitalization among diabetics patients was 17.59 days and in nondiabetics is 13.22 days and the results of the t test that means $p < 0.05$. Values of ejection fraction below 40% is more numerous in diabetics patients (Figure 3) than in nondiabetics patients (Figure 4). Corelation matrix was shown strog link beetwen diabetes mellitus typ 2 and ejection fraction below 40% (Table 2).

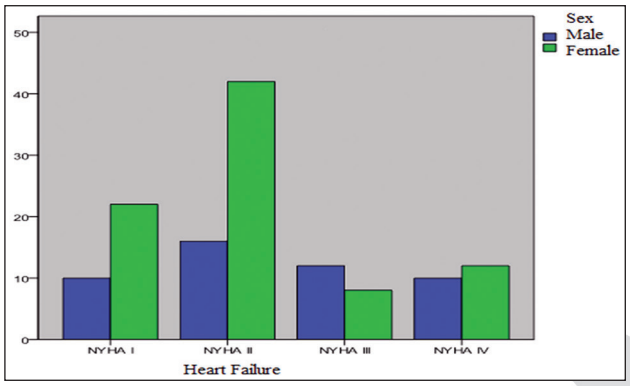

Figure 1. Heart failure in diabetics

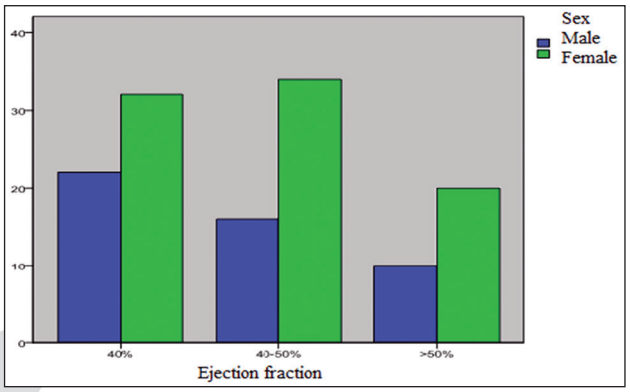

Figure 3. Ejection fraction in diabetics

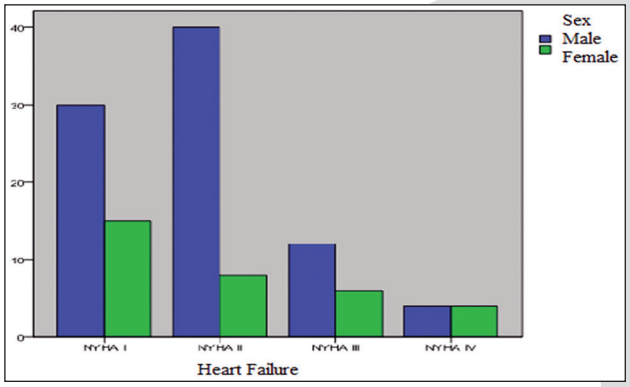

Figure 2. Heart failure in nondiabetic

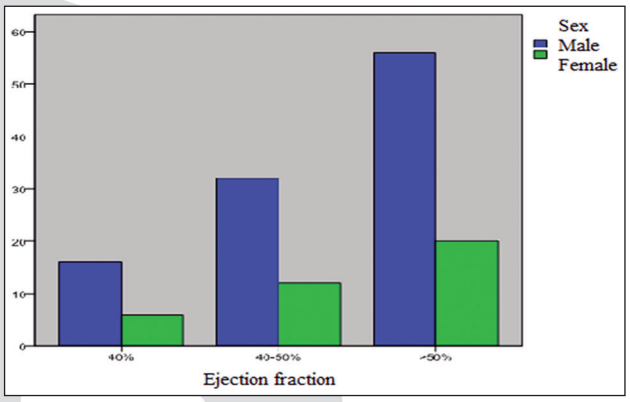

Figure 4. Ejection fraction in nondiabetic

Table 1. Correlation matrix diabetes and heart failure

Heart failure	Diabetics			Nondiabetics		
	The correlation coefficient	P value	N	The correlation coefficient	P value	N
NYHA I	0,10	0,02	20	0,11	0,6	61
NYHA II	0,21	0,01	39	0,16	0,3	40
NYHA III	0,40	0,00	30	0.21	0,5	23
NYHA IV	0,56	0,00	32	0,14	0,7	18

Table 2. Correlation matrix diabetes and ejection fraction

Ejection fraktion	Diabetes			Nondijabetes		
	The correlation coefficient	P value	N	The correlation coefficient	P value	N
<40%	0,58	0,00	54	0,33	0,57	22
40-50%	0,35	0,00	50	0,20	0,21	44
>50%	0,19	0,02	30	0,11	0,31	76

Table 3. Pearson and Spirman 'correl.between the diabetic group and the duration of treatment

		Duration of treatment	Diabetics
Duration of treatment	Pearson correlation	1	0,625
	Spirman 's rank correlation		0,623
	The level of significance		0,000
	N	134	134
Diabetics	Pearson correlation	0,625	1
	Spirman 's rank correlation	0,623	
	The level of significance	0,000	
	N	134	134

Discussion

This study is the first of a larger research of post-infarction heart failure at diabetes mellitus type 2 in this area Herzegovina-Neretva Canton. This study shows the influence of Diabetes mellitus on flow post-infarction heart failure as an important factor. In diabetics patients heart attack is a 2-3 times often than those who are nondiabetics in global popularuion. Myocardial infarction is conditioned atherosclerotic disorders of coronary blood vessels. Atherosclerotic heart disease is offten at diabetics than people in the global population. Myocardial infarction is often complicated by heart failure and a very serious complication in diabetic patients is diabetic cardiomyopathy, which reduces cardiac output at normal volumes and filling pressures of the heart.

Basically pathophysiologic occurrence of diabetic cardiomyopathy is hyperglycemia, which causes formation of cardiac damage, if we treat hyperglycemia reduces the risk of cardiovascular complications with them and diabetic cardiomyopathy.

Thanks to continued implementation of prevention research programs, and active seeking individuals with an increased risk for coronary deseases, reductions and elimination of risk factors, change of lifestyles, and the use of therapeutic - pharmacological measures have significantly reduced of morbidity and mortality trend from coronary heart deseases in developed countries. (14) Poor glycemic control can lead to chronic heart failure due to abnormal utilization of glucose by the heart muscle cells. It is known that diabetes mellitus accompanied disorder neurohormonal function with elevated levels of endothelin and proinflammatory cytokines. (15)

Conclusions

Diabetes mellitus type 2 is primarily postprandial glucose are highly significant independent factor in the development of macrovascular complications and heart attacks in latest postinfarctial clinical course and beagining of heart failure. Therefore, the European Diabetes Policy Group has set new guidelines for postprandial glucose no higher than 7.5 mmol/ L in order to reduce the risk of macrovascular incidents. (16)

Good glycemic control means prevention and delay of complications of diabetes mellitus typ 2 as microvascular like and macrovascular complications. Weight clinical course of diabetics is exclusively the result of advanced atherosclerotic lesions in the entire cardiovascular system. The cause of heart failure in diabetic patients not only diabetic cardiomyopathy, but coronary heart disease and hypertensive disease. In the pathogenesis of heart failure, which is not fully elucidated in diabetic problem microcirculation as many studies.

Risk of heart failure increases with poor glycemic control and increase the value of A1C: for every increase in A1C of 1% annual risk of developing heart failure is growing by 8%. Determination HgbA1C a set of methods for developing countries, "it is still early for a definitive position on the benefits HgbA1C of determining glucose levels in order to detect patients with diabetes, "(17) As a result of the frequency of heart failure in diabetic patients, diabetes was present in much higher percentage of studies dealing with the examination of heart failure. In this studies, diabetes regularly carries a higher risk of complications, poor outcomes and mortality and the risk is not related to patient age, possible hypertension, dyslipidemia, obesity and coronary heart disease.

Diabetes mellitus is also a vascular disease that accelerates atherosclerosis due to metabolic disorders and contributes to the development of chronic heart failure. Atherosclerosis can lead to the development of diabetic cardiomyopathy. (18)

Since the severe clinical course postinfarction heart failure in diabetic patients Compared with nondiabetic patients of the same age, this research puts imerativ as radical treatment of diabetes mellitus and unapplied inzulinoterapy wounds and Achieving the goals of good glycemic control fasting and postprandial glucose and HbA1c target values , and lifestyle changes, weight reduction, and in primary and secondary prevention diabetes mellitus type 2.

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Influences of oxLDL on ABCA1 mRNA expression and cholesterol outflow rate in U937 cells

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Abstract

Objective: To investigate the oxidized low density lipoprotein (oxLDL) on U937 cell ATP-binding cassette transporter A1 (ABCA1) mRNA expression and cholesterol efflux situation.

Methods: Human U937 cells were incubated with gradient concentrations of oxLDL (0, 25, 50, 75, 100, 125mg/L), and then dyed by oil red O to estimate the content of intracelluar lipid and detect the expressing quantity of ABCA1 mRNA by Real-time Fluorescence quantitative PCR simultaneously. Calculating the cholesterol efflux rates by using the scintillation counter to detect the amount of H^3 -cholesterol in each well cell culture plate and medium.

Results: Real-time Fluorescence quantitative PCR analysis showed that the expression levels of ABCA1 mRNA in monocytes were lower than basal line when not intervened with oxLDL, and increased drastically with oxLDL stimulation, significant difference compared with controls (P<0.01), and reached the highest level at oxLDL 50mg/L, nevertheless,continously increasing the concentration of oxLDL above 50mg/L, the expression decreased. So is the outflowing rate of intracelluar lipid. Oil red O dyeing results also suggested that celluar lipid content was the highest when intervened with 125mg/L oxLDL, and increased most obviously at 50mg/L oxLDL. Cholesterol outflow result also demonstrated that cholesterol outflow rate related with the ABCA1 mRNA expressing quantity.

Conclusion: With the increase of intervening concentration of oxLDL on U937cells, the exprssion of ABCAl mRNA represented that rising befor 50mg/L oxLDL, and then decreasing, reaching the top point at 50mg/L oxLDL. so was the change in the outflowing rate of intracelluar lipid.

Key words: U937 cells, oxidized low-density lipo-protein, ATP-binding cassette transporter 1, cholesterol metabolism, atherosclerosis.

Introduction

Cardiovascular and cerebrovascular disease resulted from atherosclerosis (AS) which plays a critical role in human health, has the highest mortality in developed countries. Recently, however, there is also a gradual increasing morbidity with years in China.The main pathological presentation of AS is the formation of plauqe in aterial vessels .Masses of basic and clinical research show that foam cell is ralated to the occurrence and development of AS.

The formation of foam cell, which is as a result of cholesterolipid engulfed and accumulated by mono-/phagocyte and SMS, is the early stage of pathological changes of AS.The latest research demonstrates that there is a close relationship between the formation of foam cells and oxidized low density lipoprotein oxLDL which can be engulfed by the external receptor of monocyte and leads to the intracellular accumulation of cholesterol ,thus lower the concentration of oxLDL around monocytes can alleviate the lever of AS[1].

In recent years, research found that, Tangier disease[2], a clinical manifestation of serum high density lipoprotein absence of hypercholesterolemia and hypertriglyceridemia early onset of atherosclerotic diseasein the ABCA1 gene mutations, resulting in loss of function of the ABCA1 protein coding, EFFLUX lipid reducing, leading to intracellular lipid accumulation, the formation of atherosclerosis, a typical cell - foam cells. Research [3] also shows some ralationships between ABCA1 the outflowing transporter of intracellular free lipid and phosphorid and the formation of AS, but in foam cell formation process,in order to reverse the

foam cells showed persistent high expression of its main protein mediated lipid outflow is unclear,we conduct this research to discuss the quality-effect relationship between gradient concentration of ox-LDL and the expression of ABCA1 mRNA ,and analyze the relevance between cholesterol outfow and the expression of ABCA1 mRNA .In such a way,we want to get acquaint with the metabolic features of choleterol in monocyte and provide the mentality to the study of preventive strategies and the mechanism of AS.

Materials and method

Materials

Human monocyte cell line-U937 were purchased from Shanghai Cell Biology; RPMI1640 medium were purchased from Thermo Fisher Biochemical Products Co., Ltd.; Fetal calf serum were purchased from Shanghai Biological Technology Co., Ltd. Super research; oxidized low-density lipoprotein were purchased from Beijing Association of Health Biosciences; ^{3}H labeled cholesterol were purchased from sigma company; oil red O were purchased from AMRESCO U.S. companies; DEPC reagent, reverse transcription reagents box, fluorescence quantitative kit was purchased from Beijing TransGen company; RNA extraction reagent kit was purchased from Beijing TIANGEN company; PCR primers were synthesized by Beijing three Bo Polygalaceae Biotechnology Co., Ltd. synthesis.

Cell culture

The healthy U937cells were cultured with 10% fetal calf serum RPMl-1640 medium, under the condition 37°C、5％CO_2 ,and experimented at the logarithmic growing phase after subculturing .

Intervened with oxLDL

Selecting U937cells which is growing in good condition .Then replacing the medium and continuous to culture the cells before the day of intervention .So as to make sure they were in the logarithmic phase the next day. The next day again after replacing the medium adjusted to cells. 1×10^{6}/ L back . Dividing the experiment into parallel groups, each 6-hole ,seeding the well- adjusted cells into two 6-well plates with each of the volume 500ul . Adding 1mg/ml of oxLDL to per Hole in each plate board in accordance with the order ,following a final concentration of 0 mg/L, 25mg/L, 50mg/L, 75mg/L, 100mg/L, 125mg/L .Gently blending the two culture plates with the tip after intervention ,then placing them into incubator and continuously culturing for 48 hours under the condition 37°C, 50mL/LCO_2.

Dyed with oil red, detecting the expression of ABCA1 mRNA and outflowing rate of intracellular cholesterol

Dyed with oil red

Removing the cells intervened for 48h .Washing the Cell samples with PBS for three times, and then droping on the glass slide with PBS suspension. fixing with isopropyl alcohol for 1 min, staining with oil red O for 20 minutes, and then hematoxylin for 10 seconds, 10mL / L HCl separation and re-blue, and at last, mounting with water-based mounting media. Obseving under the ordinary microscope: the intracellular lipids being red, nuclei being blue.

Detection of ABCA1 mRNA expression level

Harvesting the Cells and extracting total cellular RNA with RNA extraction kit. Reverse transcriptase synthesis of cDNA after Underwenting its quantitative. reverse transcription conditions were 42 °C 30min, 85 °C inactivation for 5 minutes. Again quantitating the reverse transcriptase cDNA and then underwenting quantitative PCR determination, PCR reaction conditions were denaturation at 95°C for 30 seconds;then 95 °C for 30 seconds, 55°C for 15 seconds, 72°C for 10 seconds, 40 cycles. PCR primers were:

ABCA1(Primer1):
5′GGGTGGTGTTCTTCCTCATTACTG3′
ABCA1(Primer):
5′CCGCCTCACATCTTCATCTTCATC3′
βActin(Primer):
5′ATCGTGCGTGACATTAAGGAGAAG 3′
βActin(Primer2)
:5′AGGAAGGAAGGCTGGAAGAGTG 3′。

Ct value of Target gene mRNA expression levels were based on PCR amplification curve,βActin as an internal, using $2^{-\triangle\triangle CT}$ formula to calculate the relative amount.

Determination of cellular cholesterol efflux

1) Adding ^{3}H-cholesterol to 100mL/L fetal calf serum RPMI-1640 medium to preparation 0.5uCi/ml concentration.
2) Centrifuging U937 cells to remove the waste, planting the medium after suspension into three 6-hole cell culture plate. And then placing in cell incubator under the condition of 37 °C, 50mL / L CO_2 for 24 hours.
3) Washing the cells with culture medium that Contains 100mL / L fetal calf serum RPMI-1640, then adding RPMI-1640 medium with 0.2% bovine serum albumin to the hole in each plate board in accordance with the order by adding oxLDL, following a final concentration of 0 mg/L, 25 mg/L, 50 mg/L, 75 mg/L, 100 mg/L, 125 mg/L. Then placing them in 37 °C, 50mL/L CO_2 cell incubator for 48 hours.
4) Using scintillation counter to detect the amount of ^{3}H -cholesterol in each well cell culture plate and medium.
5) Cholesterol efflux rate = CPM / total CPM × 100%.

Statistical analysis

Repeate the experiment three times befor taking the average Ct, after then calculate the target gene expression relative to housekeeping genes by calculating the difference of target gene Ct and Ct housekeeping gene, and use $2^{-\triangle\triangle CT}$ formula to calculate the relative expression of the final result, with $\bar{x}\pm s$, undergo a linear correlation analysis between cholesterol efflux and ABCA1 mRNA expression. Use Statistical software SPSS18.0 to analyze variance and then make a Paired comparison. $P<0.05$ was considered statistically significant.

Experimental result

Geting out of U937cells interved with oxLDL for 48h from the culture fluid, and dyeing the cells washed twice by PBS with oil red, then observing under the microscope, following is the result. (Figure 1)

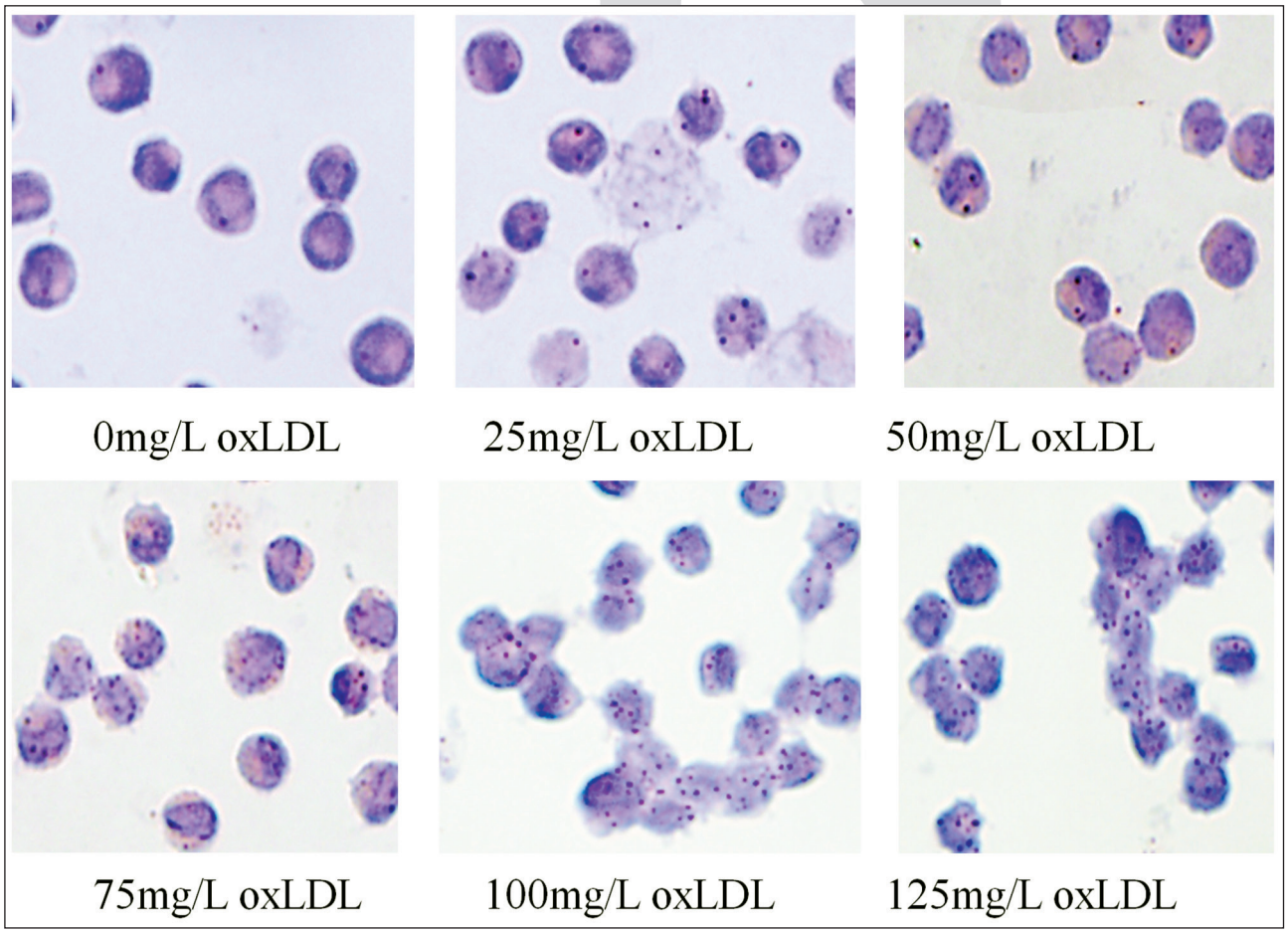

Figure 1. U937 cells were incubated with different concentrations of oxLDL and staining with oil red(10×20)

Table 1. Gradient oxLDL 48h concentration training after ABCA1 mRNA expression cholesterol metabolism (n = 3, $\bar{X}\pm s$)

oxLDL density	ABCA1	β-actin	ABCA1-β-actin	$2^{-\triangle\triangle CT}$
0(control)	21.53±1.30	22.20±0.31	-0.67±0.92	**1.00**
25mg/L	21.84±0.89	22.91±0.08	-1.06±0.6[3]b	**1.31**
50mg/L	21.30±1.40	23.46±0.70	-2.15±1.[45a],b	**2.81**
75mg/L	21.98±0.45	23.09±1.15	-1.16±0.76[b]	**1.35**
100mg/L	21.82±0.54	22.73±0.33	-0.91±0.54[b]	**1.18**
125mg/L	22.30±0.64	22.57±3.47	-0.27±0.67	**0.76**

Contrast with controls, [a]P<0.05; with 125mg/L [b]P<0.05.

The expression of ABCA1 mRNA after U937 cells intervened with Gradient concentration of oxLDL for 48h

There is a significant difference (*P*<0.01) of The expression of U937cells ABCA1 mRNA between the cells with and without intervening with oxLDL. It was at a low lever when without the stimulation of oxLDL. When increasing the dose of oxLDL, we obseved that the expression reached the peak at a 50mg/L, compared with the controls 2.81 times(*P*<0.05), after then, decreased and got a much lower lever than without intervening at the 125 mg/L. Table 1.

Efflux rate of cholesterol

There was a certain outflow of cholesterol in oxLDL stimulated U937 cells, but the outflow rate was low (7.08% ± 0.16%). With the improving of the oxLDL concentration, the flow rate began to increase significantly, and got the peak at 50mg/L oxLDL concentration, up to (16.29% ± 0.31%), with significant difference compared with control (*P*<0.01). Then as the concentration of oxLDL increase its flow rate began to reduce, and reached minimum at 125mg/L oxLDL concentration. Table 2.

Table 2. Gradient oxLDL 48h concentration training after cholesterol effux (n=3, $\bar{X}\pm s$)

oxLDL density	cholesterol effux rate
0 (control)	7.08%±0.16%[bcde]
25mg/L	10.01%±0.24%[acde]
50mg/L	16.29%±0.31%[abde]
75mg/L	11.20%±0.44%[abce]
100mg/L	8.19%±0.38%[abcd]
125mg/L	6.84%±0.28%[bcde]

compared with the control, [a]P<0.01; compared with 25mg/L, [b]P<0.01; compared with 50mg/L, [c]P<0.01; compared with 75mg/L, [d]P<0.01; compared with 100mg/L, [e]P<0.01.

The ralation between ABCA1 mRNA expression and efflux rate of cholesterol

Pearson correlation test between ABCA1 mRNA measurement results and the cholesterol efflux rate showed that cholesterol efflux rate and ABCA1 mRNA expression correlated (r=0.384 Figure 2).

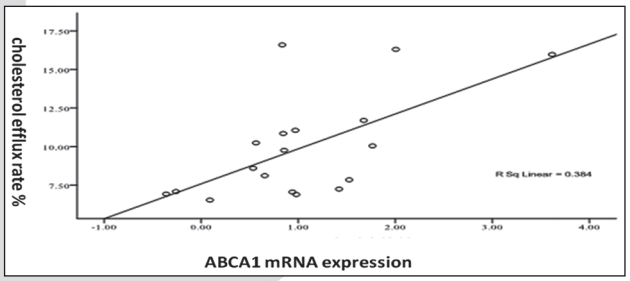

Figure 2. Relationship between ABCA1 mRNA expression and cholesterol efflux rate

Discussion

The pathological base of AS is the formation of foam cell which is the result of the lipid engufed by mono-phagocyte. The main process of AS is that oxLDL interacts with monocyte, then activates and up-regulates the ralated membraneous protein which mediates the phagocytosis ofoxLDL. However, the mass it engulfed depends on the concentration of oxLDL encircled the cell. Intervention gradient concentration oxLDL, U937 cells, we also found with the cell the surrounding oxLDL intervention increasing concentration of intracellular lipid particles show gradual increase. That is to say in certain extent when the oxLDL concentration enhances around the cell, the lipid engulfed inside the cell is also increasing, when it accumulates more and more, beyond the tolerance, foam cells is being .we may draw a conculusion that the formation of foam cell not only has

correlation with the concentration of oxLDL, but also the involved membraneous protein.

ABCA1 discovered in recent years is a kind of transmembraneous protein ralated with the formation of AS. Both clinical and animal experiment confirm that the mutation or defection of ABCAl gene leads to the decrease of serous anti-AS HDL lever ,thus giving rise to the excessively intracellular lipid accumulation, which is vulnerable to AS with the classic cell- foam cell. Human Tangier disease[2,4], main manifestation such as cardiovascular disease, an extraodinary low lever serum HDL and abnormal precipitation of intracellular cholesterol in peripheral cells, is caused by the defection of ABCAlgene. A research done by Lawn[5] mainly on patients deficient of ABCA1 gene shows that heterozygote's RCT pathway is impaired. Correspondingly some perceptible changes come into being, for instance, decreasing of serum HDL, increasing the risk to CHD, reducing outflow of intracellular cholesterol. They[4] also point out that the expression of ABCA1mRNA increasing among phagocytes in AS plaque, may be a enhanced reaction to the overload of lipid for phagocytes. We also observed in the experiment, the outflow of intracellular cholesterol was positively correlated with expression levels of ABCA1 mRNA, indicating that ABCA1 involved in cellular cholesterol efflux. AS plaque showing high expression may play the role of anti-AS, but greater than the outflow of lipid within the swallow, and eventually lead to the formation of of AS.

Human ABCA1 gene,whole length 149kb, including 50 exons and 49 introns locates at 9q31[6]. the anti-AS function relies on ATP powering for transfering multiple intracellular substates, especially the redundant intracellular cholesterol. Much more cholesterol could be tansfered out of monocytes, if gene expression increases, and this process achieved by reverse cholesterol transport RCT[7-8]. The process[9] is: Surplus intracellular cholesterolipids are hydrolyzed into free cholesterol catalyzed by neutral cholesterolipidase, and then the free cholesterol gets out of the cell and locates on serum HDL mediated by ABCA1. Through Re-esterification, tansport and distribution, HDL-CHOL complex, is finally transported to liveror adenal gland where synthesized into bile and steroids. It is RCT that acts as the protagoist in defrothing, and decides the prognosis of foam cells, which functions as the part of anti-AS .It has been verified by ABCAl transgenosis mice[10] that overexpression of ABCAl could boost the outflow of cholesterol and inhibit the formation of AS plauqe, which cuts down 65% aortic AS. Improve human nuclear/macrophage ABCA1 expression can reduce or reverse the artery AS happen?

By investigating the expression of ABCA1 mRNA located on monocytes' membrane which intervened by gradient concentration of oxLDL, we discovered that: when stimulation was not more than the dose 50mg/L, it increased as the dose rised up (up to 2.81 times compared with the control at the 50mg/L oxLDL stimulation), which was in accordance with articles that reported a peak value at 50mg/LoxLDL stimulation. Moreover, oil red O staining results suggested that the quality of intracellular lipid is not obviously rising up when cells were at the 50mg/L oxLDL stimulation. However, it increased significantly at the 75 mg/L oxLDL stimulation. Cholesterol efflux rate measured at the same time also suggested that cholesterol efflux rate related to the expression of ABCA1 mRNA, the highest (16.29%±0.31%) outflow rate happened at 50mg/L concentration of oxLDL. Then as the concentration of oxLDL increased its outflow rate began to reduce, and reached the minimum at 125mg/L oxLDL concentration (0.76 times the control). The results above suggested that increasing ABCA1 could inhibit formation of foam cell and ABCA1 might be the therapeutic targets for preventing and reversing AS.

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Immunohistochemical study of cholecystokinin, substance P and leucine-enkephalin - neurons morphology in the human inferior parietal lobule cortex

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Abstract

This investigation has been provided in order to find out is there any particular connection between the human neuronal body shapes and their immunoreactivity. Following neuropetides were examined: cholecystokinin (CCK), substance P (SP) and leucine-enkephalin (L-ENK). L-ENK -containing neurons were distributed in all the six cortical layers. In the results we found: the largest somata were obtained among SP positive neurons: average diameters (± SD) were: longer diameter 44.93 ± 15.69 mm, shorter diameter 18.16 ± 3.77 mm. One-way analysis of variance (O-ANOVA) revealed the highly significant difference among longer axis of immunopositive neurons (p=0.002). Most of the detected neurons (25%) were localized in lamina II of the LPI cortex. The least populated layer was lamina I (less than 10% of all immunoreactive neurons). The highest variability is gained inside CCK immunopositive neurons: Cajal-Retzius neurons were obtained, as well as inverted pyramidal cells, bitufted, multipolar non-pyramidal and fusiform neurons.

Key words: Parietal cortex, neuropeptides, neuron laminar distribution.

Introduction

Inferior parietal lobule (IPL) consists of two gyri: angular (AG) and supramarginal (SG). According to Brodmann's division, its cortex belongs to two areas: 39 (AG) and 40 (SG). Further parcellation provided by Eidelberg and Galaburda [1] demonstrated that cortex of IPL could be divided into five cytoarchitectonic areas, out of which three belong to AG, and remaining two belong to SG. Parietal cortex can be recognized by the presence of layer IV. In some parts of parietal cortex layer IV is very prominent. These densely granulated regions are organized in few islands and surrounded by regions of thinner layer IV, which are called disgranular cortex [2]. Specially formed layer IV is typical for parietal cortex and exists only in humans and primates [3].

The volume increase of parietal lobe characterizes particularly human brain. Development of parietal lobus partially depends on the influence from external stimuli [4]. Human IPL, as other parts of the heteromodal association cortex, according to Fredrickse and his associates [5], was reported to have sexual differences in cortex volumes. They stressed that male left IPL was more voluminous than in females and that left-right asymmetry was more prominent among male subjects. Heteromodality of IPL association cortex is confirmed by a vast number of different investigations, mostly dealing with regional blood flow changes (rCBF) in various states, diseases and disorders. It is widely accepted that IPL integrates somatosensory and visual stimuli. For example, naming animals test activated the rostral part of IPL cortex [6] and significant increasing of rCBF after cutaneous and intramuscular pain stimuli was obtained in the region of Brodmann's area 40 [7]. In the memory examination study, provided by positron emission tomography (PET), a direct comparison of conceptual and perceptual tasks showed that conceptual tasks activated medial and lateral left hemisphere in frontal and temporal regions as well as the la-

teral aspect of bilateral inferior parietal lobule [8]. Patients with lesion parts of IPL had inattention of the opposite body part [9].

Last few decades of the 20th century could be also named as "transmitters decades", due to the rapid development in technologies indispensable for the investigation of many aspects of neurotransmitters in neurosciences. Our attention was turned to several of them:

Cholecystokinin (CCK) is a peptid that consists of 33 aminoacids, but in central nervous system it is active as terminal carboxy polypeptide [10]. The highest level of this peptide, measured by radioimmunoassay, is in caudate nucleus and than in cerebral cortex. CCK is a peptid which most represented in cerebral cortex [11] including IPL [12]. Furthermore, the CCK-A receptor stimulates dopamine release in the posterior parts of nucleus accumbens and its gene was mapped to 4p15.2-15.1 with the dopamine receptor 5 gene [13].

Leucine enkephalin system (L-ENK) belongs to group of opioid peptides. Occurrence of this pentapeptid is far less than in other parts of cortex, unlike Met-Enk which is twice as big as L-ENK in the parietal region [12]. It is believed that this system has a possible role in limitation of intensity and duration of seizures, acquired so far as a response on the experimental animals [14].

Substance P (SP) is a peptide consisted of 11 aminoacids that was discovered 1931 [15]. It represents primary sensory afferent transmitter in the spinal cord. In Alzheimer's disease drastic decrease of number of neurons has been found, especially in parietal cortex [16].

The first two of mentioned, transmitters of primary gastrointestinal origin were reported to be significantly reduced in schizophrenia. Neuropeptide deficit was more pronounced in temporal and frontal lobe of schizophrenics [17].

The aim of our study was to determine the morphological aspect of neurons containing CCK, L-ENK and SP in the inferior parietal lobule, i.e. to observe their distribution through cortical layers, measure diameters of pericarya, maximal diameter of their axonal and dendritic branches, in order to obtain potential similarities and differences in their characteristics.

Material and methods

After ethics committee of Medical faculty of Belgrade approval, seven human brains of both sexes without neurological or psychiatric diseases or disorders (3 male and four female) were examined. Postmortal interval was from 3 to 6 hours. The brains underwent perfusion with Zamboni fixative (0.4M phosphate buffer, 4% paraformaldehyd and picric acid) 3-6 hours after death and put in the same solution for 7 days. After that blocks of AG and SG were isolated from hemispheres and postfixated 3 days, and then immersed into 30% sucrose solution for 48 hours. They were cut serially into frozen sections of 40 um thickness. The slices were completely washed from fixative with 0.1M phosphate buffer 5 times during 2 hours. Free-floating sections were permeabilized with 0.5% Tritron X-100 solution overnight and than we made block from endogenous peroxidase using 3% H_2O_2 during 10 minutes.

The slices were incubated in 10% normal goat serum (Miles Lab., Napperville) for 1 hour at room temperature and after that the slices were incubated for 48 hours at 4C in primary antibodies of various dilutions:

- Cholecystokinin (1 : 6000) (from coresponding laboratory)
- SP-1 (1 : 20000) (R. L. Eskay, Bethesda, USA) (15)
- Leu-ENK (1 : 4000) (from coresponding laboratory)

The characterization of the antisera used from coresponding laboratory was described formerly [18,19]. After rinsing in phosphate buffer sections were incubated in 1: 500 biotinylated antirabbit IgG solution, and than in biotinylated avidin-peroxidase complex (1: 250) (Vector Lab., Ca) for 1 hour at room temperature. Between incubation steps sections were washed in three changes of 0.1M PB. After the last wash in PB, sections were further rinsed in 50mM Tris-HCl buffer (pH 7.6).

Sections were developed in Tris-HCl buffer containing 0.02% 3.3 diaminobenzidine (DAB), 0.6% nickel-ammonium-sulphate and 0.02% H_2O_2 for 10 minutes, mounted and coversliped.

Immunoreactive neurons and fibers were drawn by Camera lucida (Olympus) and photographed on the light microscope (Olympus).

Palkovits and Fischer [20] method was applied for determining lamination of IPL cortex

Results and discussion

The lowest appearance we had in SP immunopositive neurons: only 13.5% of the observed neurons. L-ENK -containing neurons were distributed in all the six cortical layers. No presence of SP or CCK was detected in the layers V and VI. (Figure 1.)

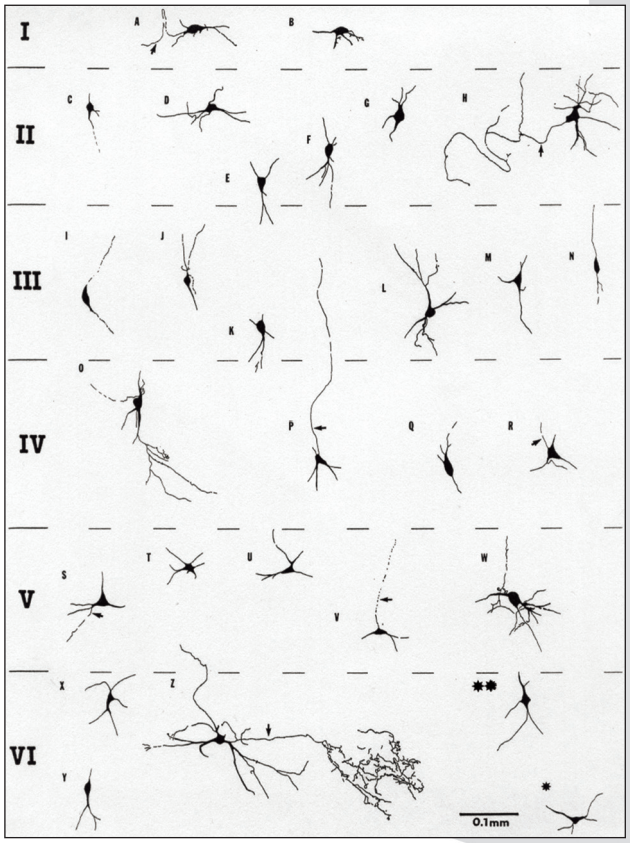

Figure 1.

The largest somata were obtained among SP positive neurons: average diameters (± SD) were: longer diameter 44.93 ± 15.69 mm, shorter diameter 18.16 ± 3.77 mm (table 2). One-way analysis of variance (O-ANOVA) revealed the highly significant difference among longer axis of immunopositive neurons (p=0.002). Most of the detected neurons (25%) were localized in lamina II of the LPI cortex. The least populated layer was lamina I (less than 10% of all immunoreactive neurons).

CCK

CCK positive neurons were found in I-IV IPL cortical layers, especially in layer II and III.

According to classification using Golgi impregnation method the following types of CCK positive are occurring in IPL cortex:

Both types of Cajal-Retzius (CR) neurons, large and small one, were identified in layer I. The main feature of these neurons is that they have ovoid cell body, oriented horizontally to pial surface and two dendrites arising to the opposite sites of the cell body, which are also parallel to pial surface. These neurons are exclusively present in the superficial lamina of the layer I.

Large CR CCK immunoreactive neuron had ovoid shaped of the cell bodies with major axis 35 mm and minor axis 18 mm. Radius of dendritic arborization was 207 mm, and they have demonstrated horizontal orientation of dendritic field toward pial surface. No spines or protrusions were remarked on the dendtrites. Their spreading direction was relatively straight (Figures 2. B, D). Small CR CCK immunoreactive neuron had major diameter of the cell body about 24 mm and lesser diameter about 11 mm. Radius of dendritic arborization was 85 mm. The cell bodies of this neuron had same features in comparison to the large one, and the only differentiating parameter was the radius of dendritic arborization.

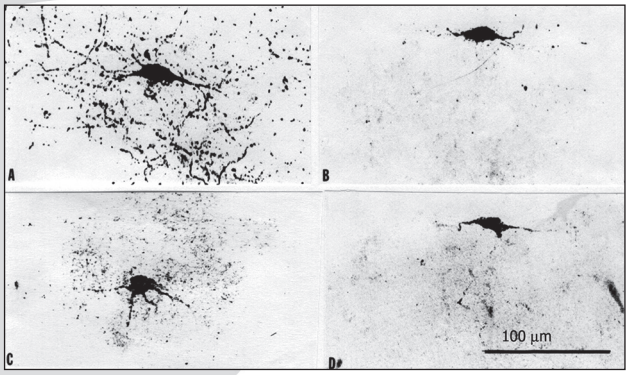

Figure 2.

Some of small CR CCK immunoreactive neurons have tortuous main dendritic branches with protrusions and spine-like processes (Figures. 2 A, C).

Inverted pyramidal (triangular) CCK neurons are characterizing the layer II. They have typical apical dendrite, which are not oriented toward pia mater. On the opposite side of the cell body are present two-three basal dendrites. Processes of CCK positive triangular cells are short and they

are smooth (without spines) (Figure 3). CCK positive cell bodies have a mean value of longer diameter 22.95 ± 5.15 mm and shorter diameter 15.51 ± 5.16 mm. Average radius of dendritic arborization was 129.87 ± 35.50 mm, and they had dendritic trees that entered only in layer III.

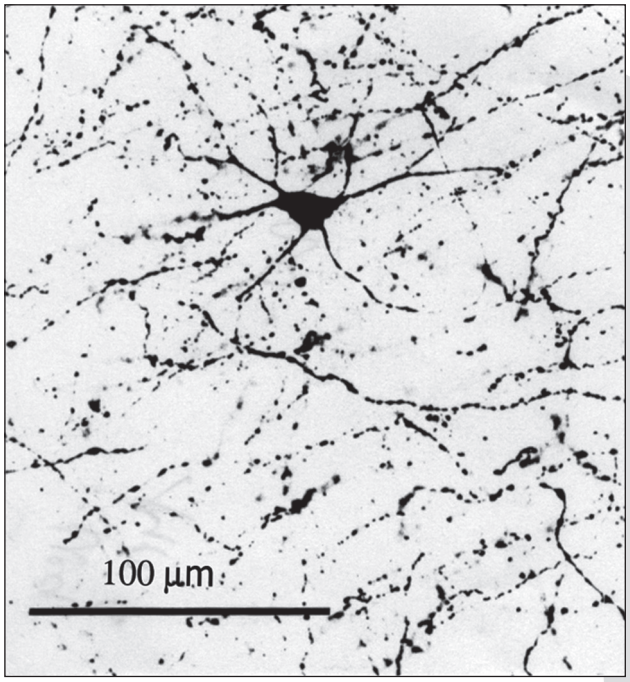

Figure 3.

Bipolar type appears to be the most frequent cells shape among CCK positive neurons. About 60% of CCK neurons are bipolar and localized in layers II and III. They have regular shaped ovoid cell bodies vertically oriented toward pial surface, and two dendrites, extended from opposite sides of the cell body. When the axons in CCK reactive cells are noticeable, they arise from the dendrites that are oriented toward white matter (Figure 4). All processes are relatively straight and without dendritic spines or protrusions. Long axis mean value in bipolar neurons was 27.51 ± 10.54 mm. Short axis mean value was 16.68 ± 4.57 mm so bipolar CCK immunoreactive neurons belong from small to medium neuronal type. Radius of dendritic arborization of these cells varied from 122 to 197 mm.

Fusiform CCK neurons were one appearance of bipolar neurons. They are situated mainly in the layer III of IPL. Characteristic of these types of neuron is slender, elongated perykaria, with predominantly vertical orientation of the dendritic field. Average values of major and minor diameters were 28.41 ± 6.38 and 13.29 ± 2.31 mm, while the radius of dendritic arborization was 188.63 ± 52.78 mm. Other features of these type of neurons are corresponding to the features of bipolar neurons.

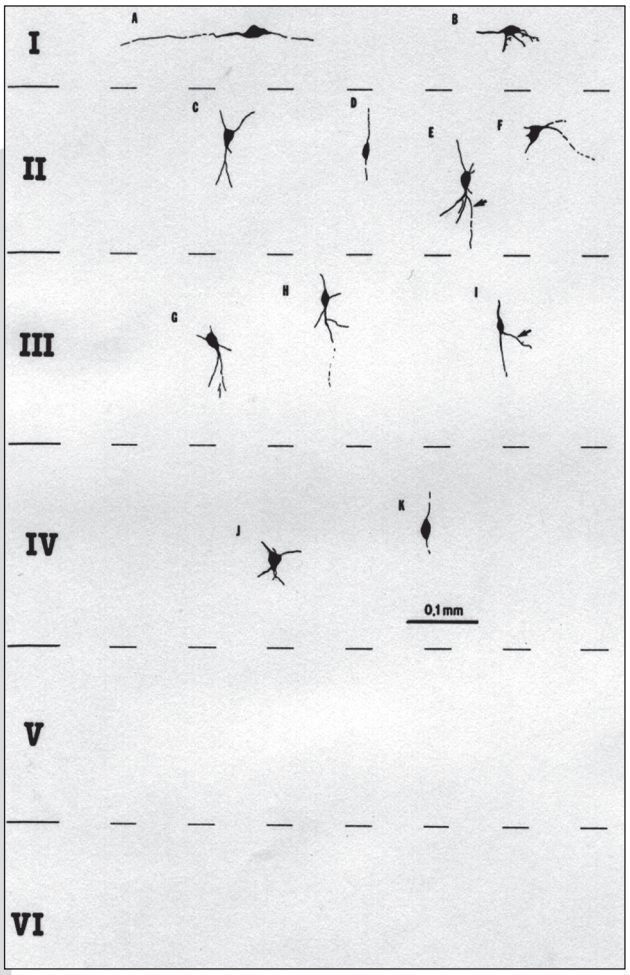

Figure 4.

Unitufted unipolar CCK immunoreactive neurons were present in layer II of IPL cortex. Generally, this type of neurons could be considered as the subtype of "double bouquet" shape somata. (Figure 4)

Leucine-enkephalin (L-ENK)

Neurons, manifesting leucine-enkephalin immunopositivity (L-ENK) were distributed in all six laminas of LPI cortex, though they were outnumbered to the letter ones (only 14 neurons in our entire sample). (Figure 5)

Six neuronal somata among obtained L-ENK cells were of multipolar type, with 3-5 primary dendrites. These neurons were spread through all cortical IPL layers, except layer I, but they were most frequent in layers V and VI. According to the

cell body major and minor diameter classification in IPL cortex existing from small to large L-ENK neurons (longer axis ranged 17 - 35 mm and shorter axis values were in the interval between 10 and 24 mm). Dendrites of these neurons were oriented in all directions and had a relatively large radius of dendritic branching (100-204 mm) (Figure 5.)

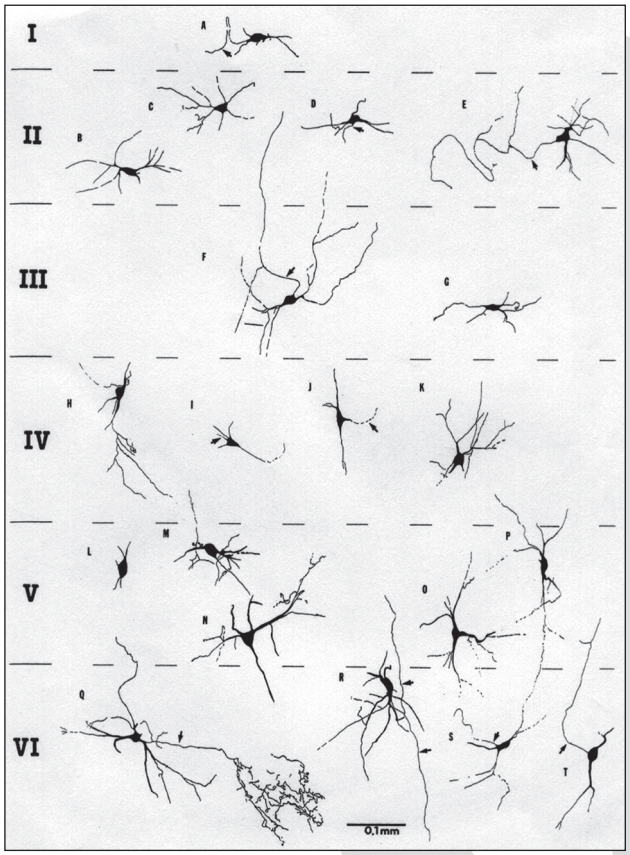

Figure 5.

Bipolar L-ENK immunoreactive neurons were located mostly from layer II to III, but they could be find in the layers IV, V and VI. Some of the L-ENK IR bipolar cells in the layer VI have unusual – horizontal position of the perykarion). L-ENK – ergic neuronal bodies have major cell body diameter from 16-24 mm and lesser cell body diameter from 11 to 14 mm. Their processes were not too long, from 80 to 110 mm.

Fusiform type of L-ENK IR neurons, as one of the bipolar varieties, was present on the border between layer VI and white matter (Figure 5.). Major cell body diameter is from 16 to 20 mm while minor diameter is from 8 to 18 mm. Dendritic trees of these cells were 88 to 109 mm long.

Unitufted unipolar L-ENK IR neurons could be found in layers II and III Dendrites of these cell types were poorly ramified, in contrast to their quite long dendritic trees (122-189 mm).

Layer V of the IPL cortex also contained a few small pyramidal cells Cajal-Retzius L-ENK positive neuron of the layer I had pyriform perikarya and short dendritic processes (Figure 5.)

Substance P

SP-1 immunoreactive neurons were rare, scattered and mostly present from layer I to layer IV. Generally, this was our smallest population of the sample and no statistical processing could be applied. The layers I and IV were poorly populated with this type of neurons (only one soma in each lamina). In IPL cortex occured:

In layer I small Cajal-Retzius neuron with simple, short two dendrites (dendritic radius 85 mm) arising from the opposite pole of the cell body. Dendrites of these cells are smooth and without processes (Figure 6).

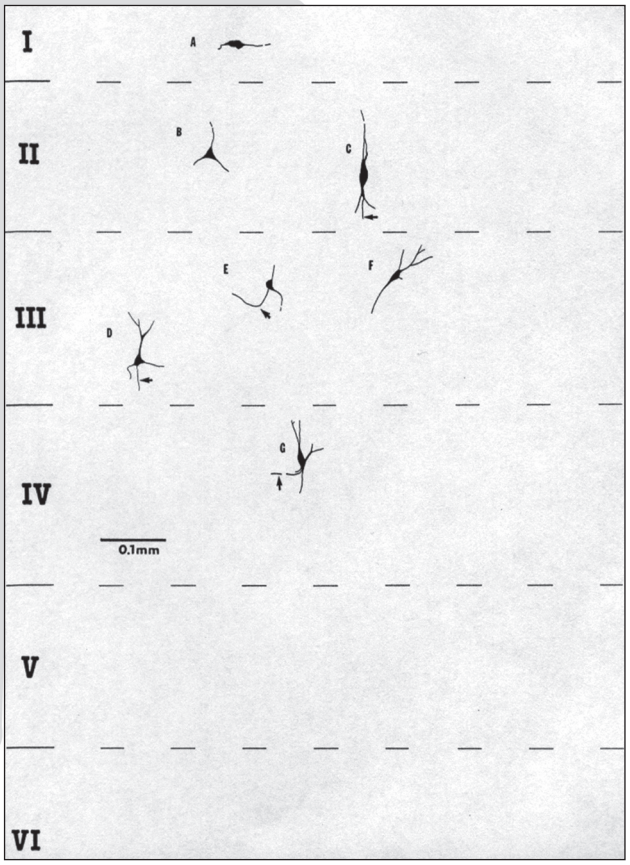

Figure 6.

Bipolar SP IR with are small (major cell body axis 12 mm and minor cell body axis 10 mm) and located in layer III.

Bitufted type SP IR cells from the layer II were oval in shape, with narrow dendritic trees (2-4 dendrites) and axon emerging from the lower dendritic tuft (Figure 7).

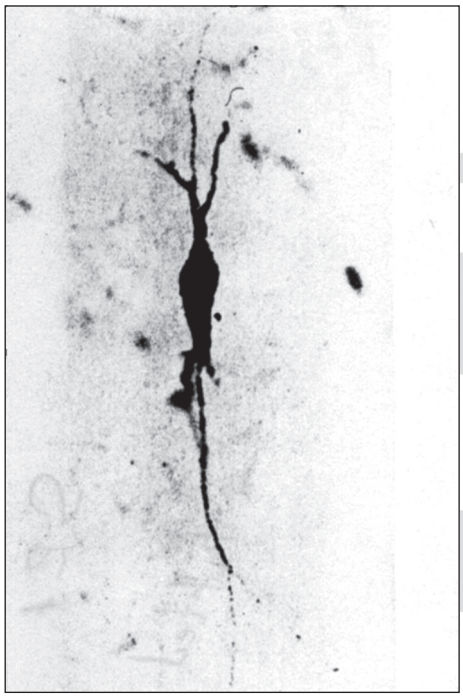

Figure 7.

Multipolar SP IR neurons have ovoid perykaria (major cell body diameter 22-34 mm and minor cell body diameter 13 to 16 mm), 3-4 smooth dendrites spread in the radiuses between 97-140 mm from the perykarion. This type was representative for the layer III of the IPL cortex Layer IV of the IPL cortex contained one small pyramidal SP IR neuron (Figure 6).

This investigation deals with the morphological variations among different types of immunopositive neurons: CCK, , SP and L-ENK, in the cortex of the inferior parietal lobule.

We used already accepted classification provided on Golgi impregnated specimens [21] and if one is willing to compare bipolar Golgi impregnated neurons to some, for example, L-ENK immunoreactive neurons, he could see no differences between. Considering the perikaryon position there was similar asymmetry as the one obtained among Golgi impregnated cells: the cells which bodies positioned in the middle of the IPL cortex had symmetrical dendritic trees, while cells which somata lied in the deeper layers had longer upper dendritic trees and vice versa.

In cortex, CCK peptide is mostly involved with two cell populations: "intrinsic neurons" which are scattered throughout cortical thickness [22] and terminal fibers projected in distant regions, like mesencephalon and thalamus [23]. Many different morphological CCK immunopositive types of neurons were obtained in our investigation: Cajal-Retzius, bipolar neurons, inverted pyramidal, unitufted, multipolar and fusiform. Majority was localized in laminae II and III. Only Cajal-Retzius neurons were obtained in the level I. This type of cell bodies was established in this investigation, while the other, with the exception of the inverted pyramidal type, have already been reported on the animal material [22]. According to biochemical analysis CCK peptide concentration in cortex decreases from frontal to occipital pole. Level of this peptide was, roughly, about 0.3 ng/mg tissue [12]. Biochemical analyses showed that CCK was a transmitter with predominantly cortical distribution, although its presence could be noted elsewhere in the central nervous system, often in co-localization with the GABA-ergic neurons [24,25]. Some experiments, reported by O'Connor [26], demonstrated that intra-accummbens CCK decreased ventral pallidal GABA release, but together with dopamine, it increased ventral striopallidal transmission, thus playing a respectable role in possible antipsychotic regulation. It was also suspected that CCK-A receptor gene might be involved in pathogenesis of auditory hallucinations in schizophrenia, according to the results of Wei and Hemmings [27]. In schizophrenics, who poorly answered to haloperidol therapy, a negative correlation of CCK in the peripheral blood mononuclear cells and Scale for the Assessment of the Negative Symptoms baseline scores was revealed, suggesting that lower basal values of CCK are supporting the appearance of the negative symptoms in schizophrenia [28].

Quite opposite to the excitatory transmitters, CCK and substance P, stands leucine-enkephalin (L-ENK) system, for which it has been stated that its concentrations in many structures of the brain raises after induced, epileptic-like, convulsions on the experimental animals [29], but, in general, its concentration in brain is low [30]. Distribution of L-ENK neurons, obtained in this investigation, might indicate its role in general regulation of ne-

uronal transmission, probably with inhibiting influence. Our opinion is strengthened by the fact that L-ENK concentration rose in hypersomnic patients [31] and that it plays an inhibiting role to the cortical and reticular formation activities.

Concluding this discussion, we can say that the morphological variations in shape of the neuronal bodies which manifested immunoreacitive labeling on three groups of neuropeptides, CCK, SP and L-ENK, are indicating their involvement in variuos roles, in which cortex of the inferior parietal lobule takes part. This complexity of cortical functions is far from being entirely investigated; moreover the action of each neuronal group, divided either by the shape or immunopositivity, is yet to be discussed.

Acknowledgements

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Correlation between smoking and oral mucosa - findings in student population in Sarajevo

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Abstract

There are 1.2 billion smokers in the world, today, according to estimates by the World Health Organization. It has established a strong correlation between smoking and the occurrence of certain diseases, determined by a number of scientific studies. It has estimated that 30% of adults smoke, and more than 5 million people die of the effects of tobacco smoking. There is a great danger for human health from consequences of smoking and its influence on all organs and organic systems in the body. The oral cavity, the first in the body, is exposed to the impact of tobacco smoke. A series of changes occurs in the oral cavity in people who smoke tobacco. The changes usually affect the entire mucosa of the oral cavity, but they are most intense on the mucosa of hard and soft palate. Nicotine is the most important ingredient in tobacco and tobacco smoke, which acts directly on the digestive, respiratory, cardiovascular and nervous system as well as on the immune system. Signs of poisoning of the digestive system occur at an early stage as nausea, vomiting and diffuse abdominal pain. Signs of poisoning of the cardiovascular system are reflected in the pallor of the skin and pink lips, racing heart, chest pain, headache, and increased blood pressure. Signs of the poisoning of the nervous system are demonstrated in the early stage, as : headache, dizziness, uncoordinated movements, excitement, impaired vision of sight and muscle spasms. Smoking repercussions on the immune system are the reduced number of lymphocytes and impaired neutrophil function in the smokers, such as the impaired production of anti-inflammatory cytokines and immunoglobulins. This finding is the result of the local, suppressed immune response. Smoking increases the secretion of adrenaline and noradrenaline, which leads to vasocontstriction of blood vessels. There are 69 carcinogens identified in tobacco and tobacco smoke. There are 11 known carcinogens to humans. Tar is carcinogen and it occurs when nicotine and water are deducted from the particles of tobacco smoke. The main carcinogens are polycyclic aromatic hydrocarbons which the result of incomplete burning. Beside nicotine and tar, the following carcinogens have been identified: carbon monoxide, carbon dioxide, nitrosamines, aromatic amines, butadiene, benzene, formaldehyde, actaldehide, hydrocyon etc. The effect of smoking on oral health has resultaed in a number of changes to the oral mucosa, in the form of white lesions, and the most common of them are candidiasis, oral lichen planus, oral leukoplakia, morscatio mucosae oris, palatis nicotinic. It is considered that smoking is the leading cause of bad breath. Tar from cigarette smoke causes yellow to brown discoloration of teeth. Carcinoma of the oral cavity is the most severe change in the oral mucosa which is caused by smoking. Carcinoma of the oral cavity accounts for 3% of all cancers and it is one of the leading causes of death. Tobacco smoke contains more than 400 chemical compounds that act on the cells of the oral mucosa, causing their malignant transformation. More than 80% of patients with oral cancer are smokers. Smokers have a 5-7 times greater risk for developing oral cancer in comparison with nonsmokers, and that risk increases with the number of cigarettes smoked per day. At the time of diagnosis, in 50% of patients , the disease is in advanced stages when the treatment options and prognosis are unfavorable. The risk of developing cancer of the oral cavity, in patients

who have stopped smoking equates to 5-10 years at risk with non-smokers. Smoking tobacco is the legal toxicomany, the oldest in the world. Health effects are reflected in all social, gender and age groups. There are sufficient data to confirm that measures in the general health education should be taken to reduce smoking and its consequences. The approach to this problem is multi-dimensional and that problem should be prevented by educational programs where education about the dangers of smoking should be an integral part .

Key words: smoking, oral mucosa.

Introduction

The adverse implications on the oral mucosa are numerous in nicotine addicts. A young person has halitosis, yellow teeth and fingers, permanent body and clothing odour, premature facial wrinkles and rugged, harsh, raspy voice, after a year of smoking. Tobacco and tobacco smoke affect the oral mucosa, respiratory and digestive system, i.e., the organism as a whole.[15, 18] The cheek mucosa shows opalescent changes with whitish deposits in the smokers, rough dry and it is susceptible to damage. A secondary infection with Candida albicans is common in the patients addicted to smoking.

Thermal effect of nicotine on the oral mucosa is clinically manifested by erythema, pain and frequent interruptions in the continuity of the oral mucosa. The changes with the bad breath, and decreased sense of taste and smell, dominate on the tongue papillae. [9, 10, 21]

Objective of the research

We intended to establish the existence of white lesions on the oral mucosa and determine a correlation between smoking and the look of the oral cavity lining.

Materials and methods

This is a clinical, applicative and controlled study with experimental- prospective character and randomly selected sample. The study was conducted at the Public Institution of the Sarajevo Canton of the Students health care Institute, at the University of Sarajevo. The students were divided into two groups: 60 smokers in the first group and 60 nonsmokers in the second group.

The groups were homogeneous in terms of age, sex, length of tobacco consumption. Their age was from 18-26 years.

During the inspection checking of oral cavity, we made photos of white lesions as documentation and put photos in a specially created questioner cardboard.

Results

The students group, aged between 18-26 years, presented a sample, and they voluntarily participated in the study, filling out the questionnaire. There were 120 students involved in the study, with an equal number of smokers and non-smokers. The smokers had significantly more frequent changes in the oral mucosa than the non-smokers. The changes in the oral mucosa were found in 50% of smokers, and in 10 % of non-smokers.

Regarding the gender, changes in the oral mucosa are significantly more frequent in female smokers - 52.6%, in comparison with changes found in male smokers - 45.5%.

Testing of differences between smokers of both sexes, according to the λ^2 test, we found a difference at level $p<0,005$ ($\lambda^2=12$) for females in comparison with males.

Table 1. Changes in the oral mucosa in smokers and nonsmokers

Sex	Smokers		Non-smokers		Smokers and non-smokers		All examined	
	N	%	N	%	N	%	N	%
Men	10	45.45	2	9.09	12	27.27	44	36.67
Women	20	52.63	4	10.53	24	31.57	76	63.63
Total	30	50	6	10.00	36	100	120	100.00

$\lambda^2=12$, $p<0,005$

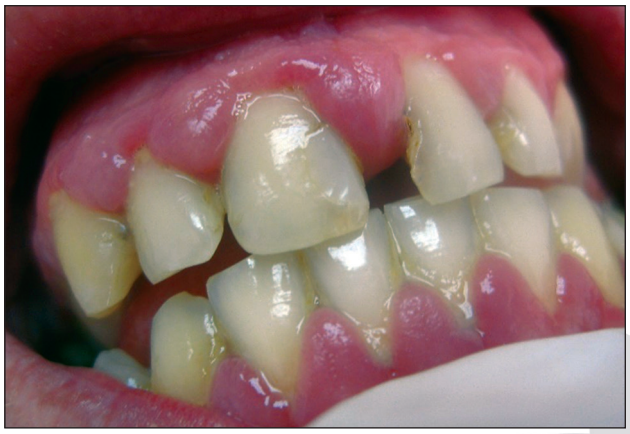

Figure 1. Changes in the gingiva of smokers (original recording)

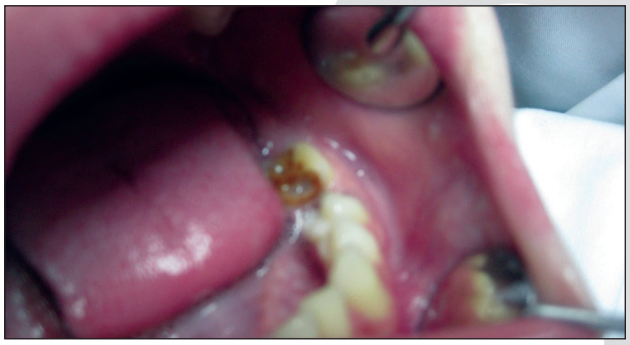

Figure 2. Changes in the oral mucosa of smokers (original recording)

Testing of differences between smokers of both sexes by the X^2 difference was found at $p<0.005$ ($X2 = 12$) for women compared to men.

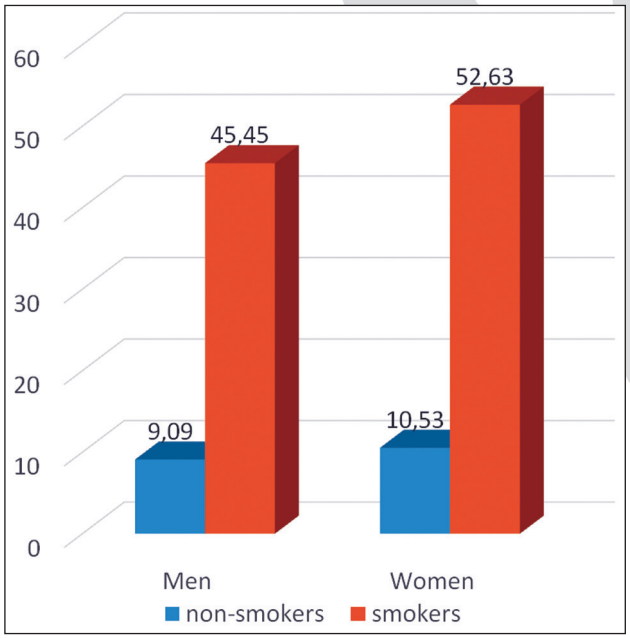

Chart 1. Changes in the oral mucosa by smoking status

Altered oral mucosa was significantly higher in smokers of both sexes, 52.6% of women, respectively, 45.5% of men, compared to non-smokers of both sexes - men 9.1, respectively, 10.5% of women.

Testing of differences between female smokers and non-smokers in whom changes were found in the oral mucosa showed statistically significant differences in prevalence at the level of $p<0.05$ ($t = 2.22$). Also, there is a statistically significant difference between smokers and non-smokers of both sexes, in total, at the level of $p<0.01$ ($t = 2.62$).

The changes in the oral mucosa were more frequently present in the smokers, in both sexes, 52.6% females, and 45.5% males, compared to non-smokers of both sexes – 9.1 males and 10,5% females.

There were significant differences in prevalence of changes in oral mucosa between female smokers and non-smokers at level $p<0,05$ ($t=2,22$), by testing the differences between female smokers and non-smokers. There was a significant difference between smokers and non-smokers of both sexes, too, at level $p<0.01$ ($t=2.62$).

Table 2. Testing of differences between smokers and non- smokers by Student t-test

Sex	t-test	t-test
	smokers and nonsmokers with changes in oral mucosa	**Total, smokers and nonsmokers**
Male	1.414116335	0.636562604
Female	2.218298214	2.891313673
TOTAL	2.618614683	9.16515139

The differences of changes in the oral mucosa were specially noticed between female smokers (62.6%), and non-smokers (10.6%). We found significant difference between female smokers and non- smokers at level $p<0,05$ (2,89) between female smokers and female non-smokers in comparison with females of hole sample.

We found a significant difference in the total examined sample (120 subjects) and the sample with changes in the oral mucosa (36 patients with changes) at level $p=0000$ ($t=9.17$).

Present differences in the samples found changes in the oral mucosa, particularly evident among female smokers (62.6%), and non-smokers (10.6%). There was a significant difference at $p<0,05$ (2.89) between female smokers and non-smokers compared to women of the entire sample.

Table 2. Testing differences between smokers and nonsmokers model t-test for each student

Sex	t-test	t-test
	Smokers and non-smokers with changes on the oral mucosa	Smokers and non-smokers total
Men	1,414116335	0,636562604
Women	2,218298214	2,891313673
TOTAL	2,618614683	9,16515139

Highly significant difference at p = 0000 (t = 9.17) was found in the total sample examined (120 subjects), and a sample found changes in the oral mucosa (36 patients with changes).

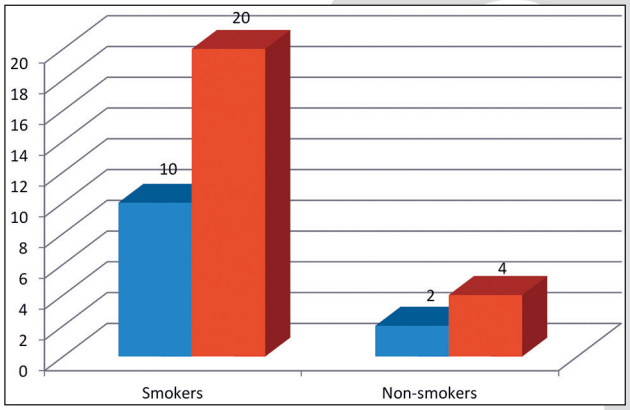

Chart 2. Changes in the oral mucosa in the total sample of respondents

Smokers and non-smokers with unchanged oral mucosa

We found opposite picture in the changed oral mucosa comparing smokers and non- smokers.

The rate of non-smokers without changes in the oral mucosa was 90.0% (in the male students 90.9% and in the female students 89.5%) compared to the smokers with changes, total 50.0% (male students 54.6% and female students 47.4%).

There were significant differences between male and female smokers and non-smokers without changes, by testing the differences with the $\lambda^{2\ test}$, at level p<0.001 (λ^2=15.457).

Smokers and non-smokers with unchanged oral mucosa

Comparing smokers and non-smokers was found exactly the opposite picture compared to smokers and non-smokers on changed oral mucosa.

Specifically, the percentage of non-smokers who had no changes on the mucosa of the total of 90.0% (90.9% of the students, and female students 89.5%) than smokers who did not have a change in the total of 50.0% (54 students , 6% and 47.4% of female students).

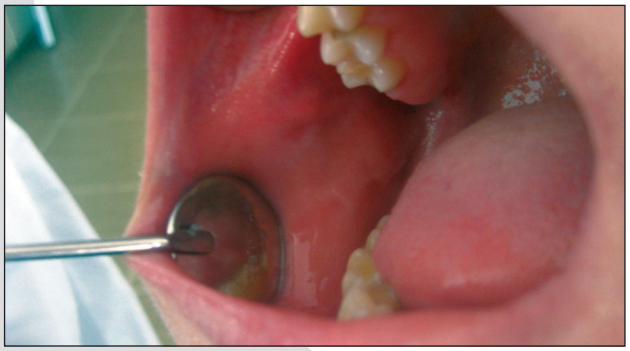

Figure 3. No changes of oral mucosa in smokers (original recording)

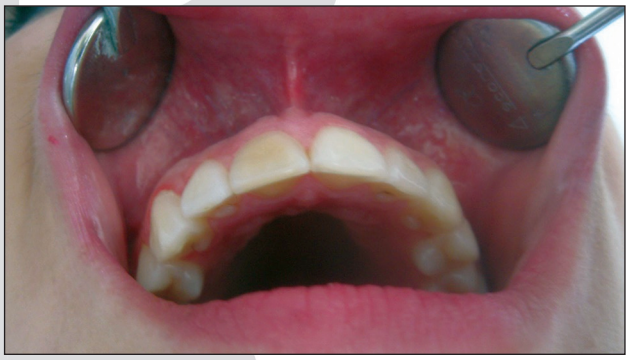

Figure 4. No changes in the oral mucosa of smokers (original recording)

Table 3. Smokers and non-smokers without changes in the oral mucosa

Sex	Smokers		Non-smokers		Smokers and non-smokers		Total	
	N	%	N	%	N	%	N	%
Males	12	54.55	20	90.91	32	72.73	44	36.67
Females	18	47.37	34	89.47	52	68.42	76	63.33
TOTAL	30	50.00	54	90.00	84	100.00	120	100.00

X^2=15.457 p<0,001

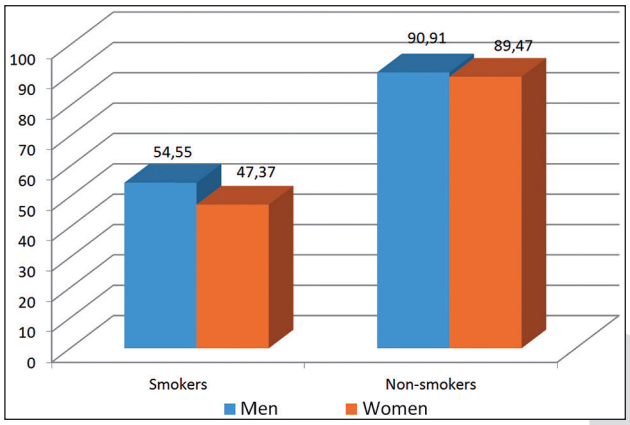

Chart 3. Smokers and non-smokers with no changes in the oral mucosa

Testing of differences between the sample of smokers and nonsmokers with unchanged oral mucosa, modeled t-test also confirmed the highly significant difference at p = 0.0000 (t = 4.0) between smokers and non-smokers of both sexes, total.

A high significant difference was confirmed between smokers and non- smokers of both sexes at level p=0, 0000 (t=4.0) by testing the differences between the sample of smokers and non-smokers with unchanged oral mucosa with the t-test.

Table 4. Testing the differences between smokers and non- smokers with unchanged mucosa

Sex	t-test smokers and non-smokers	t-test total sample
Males	-2.3092	1.788741
Females	3.26549	2.529336
TOTAL	4	3.98387

It is significant difference for females at level p<0,005 (t=3.27) and the difference for males at level p<0, 05 (t=2.31) We found the difference for females at level p<0, 05 (t=2.53) and p<0.05 (t=3.1) for all smokers and non-smokers, total, by comparing the smokers and non-smokers of both sexes with unchanged oral mucosa.

Discussion

Cekić-Arambašin A. et al (2001.) Examined the findings of yeasts in oral diseases and symptoms. [16] The study was conducted on 60 subjects were based on clinical findings and symptoms of burning mouth xerostomia established fungal infections in the oral mucosa. Fungi were isolated from pathologically altered mucosa, pelvic exam. The results showed that in only one third (1/3) found yeasts in the oral mucosa. Research, we found that the changes in the oral mucosa in 50% of smokers, compared to 10% found changes in nonsmokers. Frankovic and Topic are researching pathological changes in the oral mucosa and found changes in 57 (95%) of the fifth year dental students 25 years old. [1] Our results show that the pathologic changes in the oral mucosa are present in 50% of smokers and 10% nonsmokers. Bokor-Cousin Mary (2003.) In the article "Prevalence of oral", the results of epidemiologic studies on the prevalence of oral leukoplakia. In these studies, the prevalence of leukoplakia ranges from 0.6% to 4.6%. Variations in prevalence due to the use of different methodologies that are applicable to the type of study population and diagnostic method. Oral leukoplakia is a disease of middle age, which is common in men over 40 years of life and in women after 50 years of life. It also establishes the domination of leukoplakia in men than women, and this ratio ranges from 4:1 to 2:1. Smoking is the main risk factor for developing leukoplakia. Drinking alcohol is an important risk factor in the development of leukoplakia. For smokers who smoke a day two or more packs of cigarettes and drink more than four alcoholic drinks, the risk of leukoplakia is 6 to 15 times higher among nonsmokers and a nonalcoholic. [19]Vejle and associates (2005). Investigating the cotinine only one of a number of potentially harmful ingredients discovered in tobacco smoke. [20] Our research has shown that there is a correlation between smoking and oral cavity mucous membrane looks. In relation to gender changes in the oral mucosa in a greater percentage of women were smokers (52.63%), compared with the percentage of male smokers (45.45%). M.P.

Conclusion

Based on research of several aspects of smoking and the correlation with the findings of oral mucosa with the student population, seen as the goal of this work, we get results that show harmful effects of smoking on oral health.

Changes in the oral mucosa are significantly present in smokers compared with nonsmokers. In 50% of smokers were found to change the oral mucosa as compared to 10% of the changes found

in nonsmokers. In relation to gender, changes in the oral mucosa are significantly present in female smokers (52.6%) compared to the changes in the oral listeners smokers among men (45.5%). Testing of differences between smokers of both sexes, according to the x2 difference was found at $p < 0.005$ ($X2 = 12$) for women compared to men.

Correlation of nicotine addiction (smoking) young population and suggests screening examinations of oral mucosa and preventive education programs to kick the addiction.

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Impact of tirofiban on the C-reactive protein and cardiac marker of non-ST-segment elevation acute coronary syndrome patients after percutaneous coronary intervention

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Abstract

Aim: To observe the effects of early administered tirofiban on the C-reactive protein (CRP), cardiac marker creatine kinase isoenzyme (CKMB), troponin (TnI) and major adverse cardiovascular events (MACE) and bleeding events of non-ST-segment elevation acute coronary syndrome (NSTE-ACS) patients after percutaneous coronary intervention (PCI).

Methods: NSTE-ACS patients who received PCI were selected and randomly divided into an early administered tirofiban group (n = 29, 24 ~ 48 h before PCI) and an intraoperatively administered tirofiban group (n = 29, at the beginning of PCI). CRP, CKMB and TnI levels were detected before and 12 h after PCI respectively, and bleeding events were observed 48 h after PCI. The patients were followed up for 90 days, during which MACEs were recorded.

Results: Compared to the intraoperatively administered tirofiban group, the postoperative CRP level was not reduced, CKMB and TnI levels were not affected (P> 0.05) and MACE within the postoperative 60 days was not decreased in the early administered group. The incidence rates of bleeding event in the two groups did not differ significantly.

Conclusion: The NSTE-ACS patients receiving selective PCI did not benefit more from the early use of tirofiban than the intraoperative use.

Key words: Tirofiban, acute coronary syndrome, C-reactive protein, cardiac marker.

Introduction

Tirofiban hydrochloride, a platelet membrane glycoprotein IIb/IIIa receptor antagonist, acts on the last channel of platelet aggregation and inhibits platelet activity. Therefore, tirofiban has been clinically used for acute coronary syndrome (ACS) patients to resist thrombosis. The studies on the prognosis of tirofiban treatment and restenosis randomized efficacy and the ischemic syndrome treatment with platelet receptor inhibition are limited to the patients with symptoms and signs of instability. Tirofiban can significantly reduce the incidence rate of adverse cardiovascular event of ACS patients 7 days and 6 months after operation [1,2]. Domestic small-scale sample studies have shown that the combining domestic tirofiban based on aspirin and clopidogrel is safe and effective for perioperative ACS elderly who receive percutaneous coronary intervention (PCI) [3,4]. In this study, non-ST-segment elevation ACS (NSTE-ACS) patients were selected to explore the influences of tirofiban at different time intervals on these patients by comparing the levels of C-reactive protein (CRP), creatine kinase isoenzyme (CKMB), tropomethodsnin (TnI) and MACE.

Materials and Methods

Objects

58 NSTE-ACS patients who received selective PCI in our hospital from April 2012 to December 2012 were selected, including 36 males and 22 females, of whom the ages ranged from 45 to 67 years old with the average of 55.1. Inclusion criteria: (1) the ages of the patients were between 20 and 70 years old; (2) the patients were clinically diagnosed as NSTE-ACS, including unstable angina and NSTE acute myocardial infarction; (3) the patients received selective PCI; (4) they volun-

tarily signed informed consent. Exclusion criteria: (1) the patients with a history of gastrointestinal bleeding; (2) the patients with a history of stroke for nearly two years; (3) the patients with serious liver and kidney dysfunction; (4) the patients with acute severe infection; (5) the anemia patients with hemoglobin <100g/L; (6) the patients with platelets $<100 \times 10^9$/L; (7) coagulation dysfunction; (8) the patients who used IIb/IIIa receptor antagonist within nearly one month; (9) pregnant women; (10) cancer patients.

Study methods

58 NSTE-ACS patients were randomly divided into two groups: an early administered tirofiban group (n=29, including 19 males and 10 females, aged between 46 and 67 (56.7 ± 8.1) years old; and an intraoperatively administered tirofiban group (n=29, including 17 males and 12 females, aged between 45 and 65 (57.4 ± 9.2) years old. The two groups received preoperative routine treatment for coronary heart disease, including aspirin, clopidogrel, statin lipid-lowering drugs, β receptor blocker, ACEI/ARB and calcium antagonist, etc. In the early administered tirofiban group, tirofiban (Wuhan Hezhong Bio-chemical Pharmaceutical Co., Ltd., batch No.:20120428) was used 12 ~ 48 h before PCI. They were subjected to venous pushing injection at 10 μg/kg initially and then continuous intravenous pumping at 0.2 μg/(kg • min) until 24 ~48 h after PCI. In the intraoperatively administered tirofiban group, tirofiba was used at the beginning of PCI. They underwent venous pushing injection at 10 μg/kg at first and then continuous intravenous pumping at 0.2 μg/(kg • min) until 24 ~48 h after PCI. If case of bleeding, the dose of tirofiban was reduced until to zero.

CRP, CKMB and TnI levels of both groups were measured by sampling venous blood before and 12 h after PCI. The samples were sent to the clinical laboratory of the hospital for detection. CRP level was measured by dynamic timing nephelometry utilizing Autobio and its ancillary reagents. CKMB and TnI levels were measured by chemiluminescent microparticle immunoassay using automatic biochemical analyzer and BIOBASE reagents. Bleeding events were observed 48 h after PCI. The patients were followed up for 90 days, during which MACEs were recorded. MACEs included cardiac death, acute recurrent myocardial infarction and target vessel revascularization.

Statistical analysis

The data were statistically analyzed by SPSS 12.0 software package and expressed as (±s). The measurement data and the numeration data were compared by the t test and X^2 test, respectively. P <0.05 was considered significantly different.

Results

General information

There were no significant differences in the gender, age, platelet count, history of hypertension, history of diabetes, history of hyperlipidemia, the number of target vessel, PCI-related blood vessel, preoperative CRP level, preoperative CKMB and TnI levels of the two groups (Table 1).

CRP, CKMB and TnI levels after PCI

The levels of postoperative CRP, CKMB and TnI of the two groups are shown in Table 2 with no statistically significant differences. The results suggest that the early use of tirofiban could not reduce the CRP, CKMB and TnI levels of NSTE-ACS patients after PCI.

Bleeding event and MACE 48 h and 90 d after PCI

The bleeding events 48 h after CPI and MACE 90 d after CPI are shown in Table 3, showing no significant differences. There were 3 cases of gum bleeding and 2 cases of forearm bleeding in the early administered tirofiban group, while there were 2 and 2 corresponding cases in the intraoperatively administered tirofiban group. All MACEs in both groups were acute recurrent myocardial infarction, indicating that the early use of tirofiban did not affect the postoperative bleeding event and MACEs 90 d after PCI.

Discussion

The safety and efficacy of tirofiban in the treatment of NSTE-ACS patients have been demonstrated. Due to the risks of bleeding or even fatal bleeding, the safety of tirofiban in the treatment of elderly ACS patients has become one of the most concerns [5,6]. So far, the relevant studies on the elderly ACS patients who undergo PCI have sel-

Table 1. General information

Item	Early administration (n=29)	Intraoperative administration (n=29)
Male	19 (65.52%)	17 (58.62%)
Age	56.7±8.1	57.4±9.2
Platelet count ($\times 10^9$/L)	176.8±5.7	189.7±9.7
Hypertension	18 (62.07%)	20 (68.97%)
Diabetes	12 (41.38%)	11 (37.93%)
Hyperlipidemia	2 (6.9%)	3 (10.34%)
Target vessel		
1	16 (55.17%)	18 (62.07%)
2	12 (41.38%)	11 (37.93%)
3	1 (3.45%)	0
Lesion position		
Left anterior descending branch	19 (65.52%)	18 (62.07%)
Left circumflex branch	11 (37.93%)	13 (44.83%)
Right coronary artery	6 (20.69%)	7 (24.14%)
CRP level before PCI (mg/L)	3.71±0.91	3.32±0.94
CKMB level before PCI (ng/mL)	0.62±0.13	0.78±0.19
TnI level before PCI (ng/mL)	0.02±0.01	0.04±0.02

Table 2. CRP, CKMB and TnI lvels after PCI

Group	n	CRP (ng/mL)	CKMB (ng/mL)	TnI (ng/mL)
Early administration	29	6.42±0.81	0.98±0.62	0.18±0.15
Intraoperative administration	29	5.94±1.04	1.16±0.71	0.14±0.11

Table 3. Postoperative bleeding event and MACE (case)

Group	n	Bleeding complication	MACE
Early administration	29	5 (17.24%)	3 (10.34%)
Intraoperative administration	29	4 (13.79%)	3 (10.34%)

dom been referred. Besides, the limited studies also suffer from small sample size and undefined long-term effect.

NSTE-ACS is induced by coronary plaque instability and rupture, which result in platelet adhesion and aggregation that lead to myocardial ischemia, injury, necrosis and clinical cardiovascular events [7,8]. Tirofiban hydrochloride is a non-peptide platelet membrane glycoprotein IIb/IIIa receptor antagonist with high specificity, which blocks the final pathway of platelet aggregation by combining with the receptor to treat ACS patients [9]. In a clinical trial TACTICS-TIMI 18 study, tirofiban significantly reduced the MACEs of NSTE-ACS patients receiving PCI, confirming the impacts of tirofiban on these patients [10,11]. Lang also confirmed the efficacy of tirofiban on ACS patients [12]. However, the timing of tirofiban administration is still controversial.

First, the pros and cons of early or intraoperative administration of tirofiban were compared by the CRP levels in this study. CRP, as a common inflammatory factor produced by the liver, can reflect the body's inflammatory conditions. It is now well-acknowledged that CRP, which is involved in the entire process of coronary atherosclerosis, can reflect the prognosis of ACS patients. Lodh et al. believed that CRP is not only an indicator predicting the prognosis of coronary heart disease, but also a sign of successful treatment. Current studies suggest that tirofiban exerts an anti-platelet effect, and also plays a critical role in reducing CRP and other inflammatory factors [13]. Koc et al. reported that for high-risk diabetes patients, tirofiban significantly reduced CRP, and they thus considered it anti-inflammatory [14]. In this study, the CRP levels after PCI were increased compared to those before PCI in both the early administered

tirofiban group and the intraoperatively administered tirofiban group [(6.42 ± 0.81) mg/L, (5.94 ± 1.04) mg/L], between which the difference was not statistically significant. According to the results herein, the early or intraoperative use of tirofiban did not affect the inflammatory response of NSTE-ACS patients after PCI, inferring that it might not influence the prognosis of these patients.

Secondly, CKMB and TnI levels were used to compare the functions tirofiban in the early and intraoperative administration. CKMB and TnI levels increase during myocardial injury and necrosis, reflecting the level of myocardial injury and necrosis. PCI may squeeze plaques, leading to plaque rupture, and debris and blood clots with blood flow to the distal coronary arteries to cause microembolization, resulting in elevated CKMB and TnI levels. It has been reported that tirofiban reduced the release of CKMB and TnI in the ACS patients receiving PCI after PCI. In this study, the levels of CKMB and TnI after PCI were (0.98 ± 0.62) ng/ml (0.18 ± 0.15) ng/ml respectively in the early administered tirofiban group, and (1.16 ± 0.71) ng/ml and (0.14 ± 0.11) ng/ml respectively in the intraoperatively administered tirofiban group. There were no statistically significant differences between the two groups. Early or intraoperative use of tirofiban did not lead to obvious differences in CKMB and TnI levels in NSTE-ACS patients after PCI, and did not evidently affect the reducing of post-PCI myocardial injury and necrosis. Similarly, Arbel et al. reported that for the NSTE-ACS patients undergoing PCI, the early use of tirofiban and intraoperative use of abciximab were compared, which did not reduce the peaks and cumulative release levels of myocardial necrosis marker CKMB and TnI [15].

In addition, 90 d MACE was used to compare the prognosis of early and intraoperative use of tirofiban in this study. There were two cases of 90 d MACE in both groups without significant differences. The above results show that the early use of tirofiban can not lower the odds of MACE of the NSTE-ACS patients after PCI. According to the Meta analysis by Zhang et al., 18,438 cases of high-risk ACS patients undergoing PCI were divided into an early use of glycoprotein IIb/IIIa receptor antagonist group and an intraoperative use group. The results show that the early use of glycoprotein IIb/IIIa receptor antagonist did not reduce the incidence of death and myocardial infarction [16]. In a previous clinical trial, 8,746 cases of median- and high-risk ACS patients were selected, in which the ischemic complications in the intraoperative use of glycoprotein IIb/IIIa receptor antagonist group were not significantly more than those in the early use group. In another randomized clinical trial, 8648 NSTE-ACS patients were selected, in which the early use of eptifibatide was not superior to intraoperative use in 96 h and 90 d MACEs. The results are consistent with those in the clinical trials of Mamas et al. [17].

Tirofiban, an antiplatelet drug, may lead to bleeding event. In this study, there were 5 cases and 4 cases of bleeding event in the early use of tirofiban group and the intraoperative use group respectively, between which there was no significant difference. It is well-acknowledged that the early use of IIb/IIIa receptor antagonist significantly increased bleeding events [18], which is not consistent with the results in this study because of the small sample size herein.

In summary, for the NSTE-ACS patients receiving PCI, the postoperative CRP level was not reduced in the early administered tirofiban group. Besides, CKMB and TnI levels were not affected and 90d MACE was not decreased after PCI. In other words, the early use of tirofiban was not that advantageous, which is thus not necessary. Larger-sized randomized clinical trials are still ongoing.

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The difference in the structure of the body in women from adolescence to adulthood

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Abstract

Objective: The aim of this study was to investigate the differences in the structure of the body (body composition) between women at different chronological ages.

Methods: The sample N = 150 was obtained from a population of women who are involved in beginning recreational activities (aerobics) in two fitness clubs and workers at the shoe factory. The sample was divided into six (6) sub-samples of 25 women at the age from 18-46 years.

Results: Tracking the trend of development of dimensions of the composite structure of the body, based on the descriptive parameters of the average value of the results, in all applied variables showed progressive linear increase in results with increasing chronological age. The results of all applied variables vary depending on the age of the examinees. Applying multivariate and univariate analyzes of variance statistically significant differences in the seven (7) body structure variables in women of different age from 18-46 years of age were found.

Conclusion: It was found that the distribution of subcutaneous adipose tissue with increasing age, especially after the age of 37 tends to accumulate in the central part and the stomach, the lower limbs and back. Women could remove such negativity with lifestyles changes, among others to engage in regular, programmed physical activities, which are the healthiest way of disease prevention and health maintenance.

Key words: Women, chronological age, subsamples, variables, body composition.

Introduction

From adolescence through adulthood, to old age, a woman goes through a series of different stages, which are largely biologically determined.[1] Period of late adolescence in females is characterized by harmonization of motor and functional ability, and body composition. Taking into account the fast pace of life, exposure to various stressful situations and the effect of risk factors, there is a clear need of engagement of women in regular and programmed physical exercise in order to achieve fitness and improve health.[2] In addition, obesity, a typical consequence of the sedentary way of life and work, and also the risk factor of various diseases, most notably decreases with the different programs and education. Stress, which impairs the functioning of the organism, and pollution of the environment, which affects the quality of life, also threaten the health and survival of mankind.[3] Overeating combined with hyperkinesias and excessive nervous-emotional stresses cause the largest number of modern civilization diseases: diseases of the musculoskeletal system, diseases of the heart and blood vessels, respiratory, digestive and nervous-emotional diseases.[4] However, correction of body composition is often identified with a reduction in body weight, which is wrong. Decrease of body weight does not mean at the same time reduction of the percentage of body fat, because the reduction can also occur on the basis of reduction of muscle tissue, which is not good. The essence of most programs for correction of body composition refers to the loss of body fat while preserving muscle tissue or enlarging it. There are also cases of extremely lean persons where increase of body mass is indicated, and this is primarily due to increased muscle mass, with possible small increase in adipose tissue. American Alliance for Health, Physical Education, Recreation and Dance AAHPERD – among other components of physical fitness include body composition.[5] Body composition is the percentage of fat, muscle and bone tissue in total body weight. One of the most

popular methods for determining body composition, which is applied in this paper too, is the method of bioelectrical impedance.[6] Main objective of this research is to determine the differences in the structure of the body between women at different chronological ages with the assumption that the increase of chronological age produce changes in body composition in terms of the amount and distribution of subcutaneous adipose tissue.

Methodology

A sample of 150 examinees was obtained from a population of women who have just been engaged in recreational programs in two fitness clubs and workers at the shoe factory in that are not involved in any organized physical activity. The sample was divided into six subsamples of 25 examinees according to chronological age (table 1).

Table 1. Sample structure

Chronological age	Subsamples
18-21	25
22-26	25
27-31	25
32-36	25
37-41	25
42-46	25
Total:	**150**

Variables in this study consisted of parameters of Body Composition Analyzer "TANITA BC-540":

FAT% - Percentage of Total Body Weight Consisting of Fat).

FFM - Fat Free Mass.

TBW - Total Body Water.

BMI - Body Mass Index.

BMR - Basal Metabolic Rate.

FAT MASS - Total Fat Weight in the Body.

IMPEDANCE - Impedance.

Data analysis

Variables applied in this study were processed by standard descriptive procedures; the basic central and dispersion parameters were calculated in order to determine the function of the basic parameters of the distribution functions for the investigated area.

To determine the statistical significance of differences among the four groups of results for the investigated area was applied multivariate analysis of variance (MANOVA).

Statistical significance of the results of the morphological characteristics of the observed subsamples (divided by the criterion of chronological age) was examined by analysis of variance (ANOVA).

Results and Discussion

In table 2, the descriptive parameters of the investigated variables of the body structure for the whole sample of examinees, age from 18-46 years are shown. Obtained values of central and dispersion parameters of applied variables show normal distribution of results.

Trends of changes of the values of the applied variables - body structure

The graphs 1-7 presents trends of changes of the variable structure of the body.

Table 2. The central and dispersion parameters of the body structure (body composition) for the whole sample of subjects

Varijable	N	Min	Max	Mean	SD	Skewnes	Kurtosis
BMI	150	17.10	40.06	24.50	3.330	.618	.376
BMR	150	4954.00	8703.00	6086.00	680.30	1.146	1.147
FAT %	150	13.50	46.80	30.85	7.723	- .273	-1.246
FAT MASS	150	6.90	46.80	24.65	9.473	.597	-.436
FFM	150	21.70	65.70	47.50	4.360	.974	1.376
TBW	150	26.18	54.50	35.10	4.265	1.074	1.530
IMPEDANCE	150	296.00	767.00	570.35	63.30	- .843	.341
VALID N (listwise)	150						

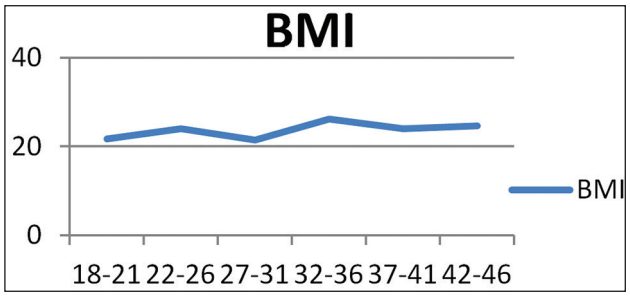

Figure 1. Changes in the values of the variable Body Mass Index

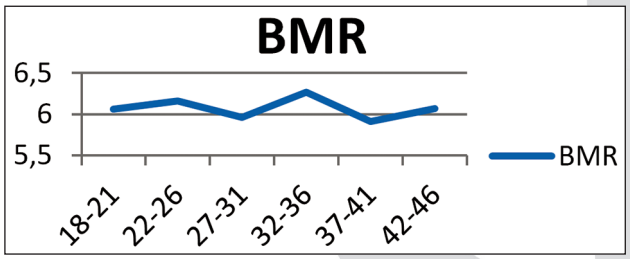

Figure 2. Changes in the value of the variable Basal Metabolic Rate

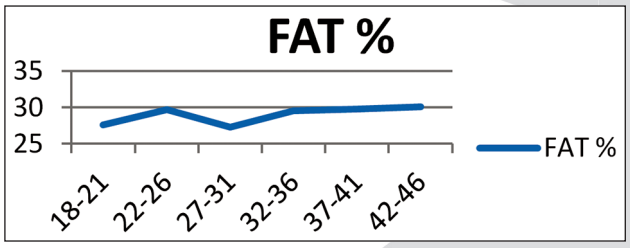

Figure 3. Changes in the values of a variable percentage of Total Body Weight Composed of Fat

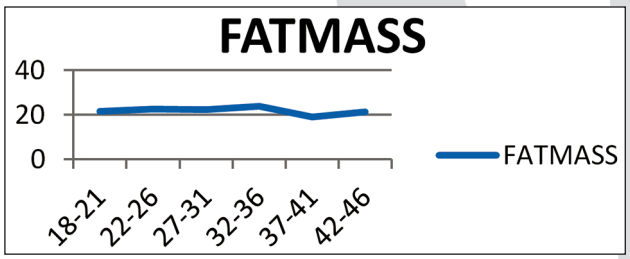

Figure 4. Changes in the values of the variable Total Fat Weight

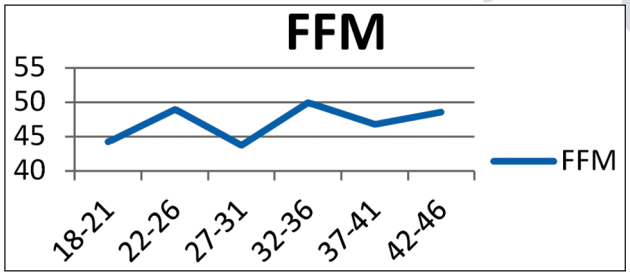

Figure 5. Changes in the values of the variable Fat Free Mass

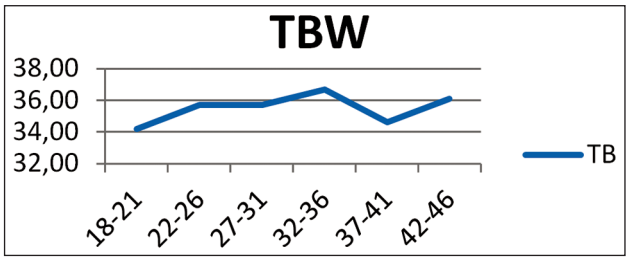

Figure 6. Changes in the values of the variable Total Body Water

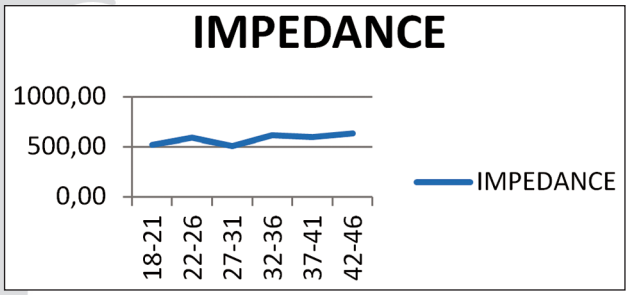

Figure 7. Changes in the values of the variable Impedance

Tracking the trend of development of dimensions of the composite structure of the body, based on the descriptive parameters of the average value of the results, in all applied variables (figures 1-7) showed progressive linear increase in results with increasing chronological age. The results of all applied variables vary depending on the age of the examinees. The period of late adolescence in females is characterized by harmonization of motor and functional ability, and body composition. Females have a significantly higher proportion of fat in the total body composition in relation to men, and the ratio of fat and fat free mass in the body changes throughout life. Also, distribution of subcutaneous adipose tissue in relation to particular body areas is changing too.[7] It was found that the distribution of subcutaneous adipose tissue with increasing age, especially after the age of 37, tends to accumulate in the central part and the abdomen, then the lower limbs and back. Similar results in a sample of women from 35-45 years received Shahan et al [8], Beissmann et al[9] in women aged 18-43 years, Hughes et al.[10] on a sample of 53 men and 78 women with a mean (± SD) initial age of 60.7 ± 7.8 y, and Korovljev et al.[11] in 49 patients 30-49 years old, and Fantin and sar.[12] on a sample of ninety-seven women and 62 men aged 71.4 ± 2.2 and 71.6 ± 2.2 years.

Tables 3 and 4 show results of multivariate analysis (MANOVA) and univariate analysis of

variance (ANOVA) of composite body structure variables (7 variables) of respondents, divided into 6 subsamples of 25 respondents in relation to chronological age.

Multivariate analysis of variance (MANOVA) revealed that the sub-samples are statistically significantly different in the composite structure of the body and that there are clearly defined boundaries between groups of subjects.

Table 3. Significance of intergroup differences in composite structures of the body

	n	F	p
MANOVA	7	4066	**.000**

Univariate analysis of variance (ANOVA) revealed significant differences in most variables, except variables: basal metabolic rate (BMR) and the total amount of water (TBW) in the body (table 4).

Table 4. Statistical significance of differences between variables of composite body structures

ANOVA	F	P
BMI	4,345	**.008**
BMR	1,467	.144
FAT %	6,335	**.000**
FAT MASS	7,349	**.000**
FFM	4,747	**.005**
TBW	1,686	.114
IMPEDANCE	3.385	**.017**

Conclusion

In this paper the differences in the body structure of the women of different chronological age were investigated and with appropriate methods, algorithms and programs obtained data on certain characteristics of the body structures of the sample, which complements the information on the development of women and the changes that occur at certain stages of their development (age periods). There were significant differences between the subsamples examined in morphological characteristics and body composition with increasing chronological age that is characterized by increasing the fat content and the characteristic distribution of subcutaneous adipose tissue. Women could remove such negativity with lifestyles changes, among others to engage in regular, programmed physical activities, which are the healthiest way of disease prevention and health maintenance.

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In vitro antitumor effects of a tradition Chinese spleen-stomach nourishing formula

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Abstract

Aim: To study the effects of a tradition Chinese spleen-stomach nourishing formula on human gastric cancer cell line SGC7901.

Materials and methods: We investigated the inhibition, invasion, apoptosis and cell cycle of the cancer cells by the formula utilizing MTT, Transwell and flow cytometry, and compared the antitumor results of the formula with those of sorafenib and analgesic antitumor peptide (AGAP) in Nanjing University of Chinese Medicine from June 2011 to June 2012.

Results: The cells treated by the formula and the other antitumor agents all became spherical, and the cell gaps were enlarged. The numbers of all the treated groups decreased. The MTT test exhibits that the formula triggered the apoptosis of 50% of the cancer cells at low concentrations similar to the conventional antitumor agents did. The Transwell test shows that the number of the formula-treated cells (67.3±18.3) that penetrated the filtration membrane was significantly lower than that of the control group (229.7±40.2) ($P<0.01$), indicating that the formula resisted to cell invasion evidently. The apoptotic rate of the formula group was significantly higher than that of the control group, particularly in the late stage ($P<0.01$), and the formula apparently reduced the number of S phase cells and increased that of G2/M phase cells.

Conclusion: The formula displayed excellent in vitro antitumor effects, which is feasible in further in vivo and clinical applications.

Key words: Gastric cancer, spleen, antitumor, traditional Chinese formula.

Introduction

Gastric cancer is one of the most common digestive tract malignant tumors (1). Approximately 10620 American people died of gastric cancer in 2009 (2). Similarly, the Chinese people are also severely threatened by gastric cancer. The 5-year survival rate of gastric cancer in China is only elevated by 5% (3) to 20%-30% (4) during the last two decades.

Although gastric cancer has not been definitely defined in traditional Chinese medicine (TCM), TCM has been devoting to dealing with the related symptoms. TCM ascribes the tendency, development, spreading and metastasis of gastric cancer to the syndrome of spleen deficiency. Many patients have already suffered from deficiencies of spleen and stomach such as anorexia, abdominal distention and diarrhea before diagnosis. Thereafter the syndrome of spleen deficiency becomes more prominent after tumor resection owing to the resulting injuries.

The TCM spleen-stomach nourishing method helps gastric cancer patients to prolong the survival duration, reduce the recurrence rate and metastasis rate, alleviate the side effects of radiotherapy and chemotherapy, and improve the quality of life. It has been preciously reported that TCM stabilized DNA and prevented DNA from the oxidation of free radicals by influencing the signal pathway targets via gene expression regulation (5). However, the underlying mechanism is still undefined. Thereby motivated, we herein investigated the in vitro effects of a spleen-stomach nourishing formula on the inhibition, invasion, apoptosis and cell cycle of gastric cancer cell line SGC7901 by MTT, Transwell and flow cytometry. Besides, we compared the effects of the formula with those of sorafenib and analgesic antitumor peptide (AGAP).

Materials and methods

General Information

All the experiments were performed in Nanjing University of Chinese Medicine from June 2011 to June 2012. The experiments have been approved by the Animal Ethics Committees of Nanjing University of Chinese Medicine and strictly performed according to the NIH guide for the Care and Use of Laboratory Animals.

Materials

Apparatus

3111 CO_2 incubator (Thermo); DMIL inverted fluorescence microscope (Leica); 5810R high-speed refrigerated centrifuge (Eppendorf); 80-2 low-speed centrifuge (Shanghai Medical Instruments (Group) Ltd., Corp.); micro sample syringe (Eppendorf); Thermo Scientific microplate reader; sterile operator (Suzhou Plan Laboratory System Engineering Co., Ltd); culture plate (Corning); Transwell chamber coated with matrigel (Millipore); FACSCalibur flow cytometry (BD).

Cells

The SGC7901 gastric cancer cells were provided by the Digestive Center Laboratory of Nanjing Drum Tower Hospital.

Reagents

RMPI-1640 culture medium (Gibco); fetal bovine serum (Hangzhou Sijiqing Bioengineering Material Co., Ltd.); trypsin (Gibco); MTT (Sigma); cell cycle and apoptosis detection kits (Fisher Scientific); TCM extract of the spleen-stomach nourishing formula (Clinical Pharmacology Laboratory, Jiangsu Province Hospital of TCM); AGAP extract (Molecular Biology Laboratory, Jiangsu Province Academy of TCM); sorafenib (Luxuriant Bamboo Chemical Tech (Wuhan) Co., Ltd., Patch No. 01109356).

Methods

Preparation of sorafenib tosylate

DMF and DMSO (1:1) were prepared into a 1g/ml solution, filtered by a 0.2 μm filter membrane, subpacked, sealed, and stored at -20 °C. The solution was diluted and added into the culture medium containing 10% FBS exactly before use. Higher than 100 μg/ml sorafenib produced floc. The DMSO content was lower than 0.01% when approximately 15 μg/ml sorafenib killed 50% of the SGC7901 cells, so the influence of DMSO can be excluded.

Other drugs

AGAP was diluted by a serum-free RMPI-1640 culture medium, filtered, sterilized, subpacked, sealed, stored at -20 °C, and transferred to the storage at -4 ℃ prior to use.

Cell culture

The culture medium in which approximately 80% of the cells were fused was discarded. The culture bottle wall was covered by adding 0.25% trypsin solution. RMPI-1640 culture medium (4 ml) containing 10% FBS was immediately added to the culture bottle to terminate digestion when the cells became spherical, the cytoplasm shrank, the cell gap was enlarged or a small number of cells were exfoliated. Then the completely exfoliated cells were centrifuged at 1000 r/min for 5 min. After discarding the supernatant, a cell suspension that was prepared by adding another culture medium was inoculated into 2-3 culture bottles, and incubated in a 5% CO_2 incubator at 37 ℃.

MTT test

The cells growing exponentially were prepared into single cell suspensions by being digested by 0.25% trypsin. The cells adjusted into the concentration of 5×10^3/mL were inoculated in 96-well culture plates (200 μl/well). Then the cell were incubated in the 5% CO_2 incubator at 37 ℃ for 24 h. The cells were untreated (control) or treated with sorafenib, spleen-stomach nourishing formula, and AGAP, respectively. The drugs were added by being diluted by 10 times. A blank well and five parallel wells were set for each group, and the cells were incubated in the 5% CO_2 incubator at 37 ℃. 20 μl of MTT solution (5mg/m) was added to each well 24 h after the treatment, and the cells were further incubated for 4 h. Then 150 μl of DMSO was added to each well after carefully discarding the supernatant in the wells, and thereafter the plates were oscillated at room temperature for 10-15 min to fully dissolve the crystals. The absorbance (A) of the solution in each well was measured at 490 nm. Cell growth inhibition rate (%) = $(1-A_{experiment}/A_{control}) \times 100\%$.

Transwell test

The cells that were digested, centrifuged and washed once-twice with PBS were re-suspended in the culture medium containing 1% FBS. The cell concentration was adjusted to 5×10^5/ml. 200 μl of the cell suspension was added to a Transwell chamber, to which was then added different drugs. Three parallel wells were set for each group. 500 μl of the culture medium containing 10% FBS was added to the bottom chamber of a 24-well plate. Then the cells were incubated for 24 h. Thereafter the adherent cells were counted under a microscope. The cells were washed twice with calcium-free serum, fixed in methanol and air-dried properly after discarding the culture medium. Then the cells were stained by hematoxylin for 20 min, from which the unmigrated cells were wiped gently by a cotton stick. The cells were then observed and counted under a 400× microscope after being washed three times with PBS.

Apoptotic test

The culture medium was transferred to a marked 15 ml centrifuge tube and washed twice with PBS after the cells were treated for 24 h, and the PBS washing solution was also transferred in the corresponding tube. 1 ml of 0.25% trypsin solution was immediately added to the culture bottle, and the cells were incubated in the 5% CO_2 incubator at 37 ℃ for 1-2 min, after which the digestion was terminated when the cells became spherical, the cytoplasm shrank, the cell gap was enlarged or a small number of cells were exfoliated. Then the cells were completely exfoliated by being blown repeatedly. Subsequently, the exfoliated cells and the solution were transferred to a 15 ml centrifuge tube and centrifuged at 1000 r/min for 5 min. After discarding the supernatant, freshly prepared and pre-cooled PBS was added to resuspend the cells. Then the uniformly mixed cells were centrifuged at 1000 r/min at 4℃ for 5 min and resuspended after discarding the supernatant. The procedure was repeated. Then the apoptotic test was performed within 1 h according to the instructions of the kit.

Cell cycle

The cell concentration was adjusted to 2.5×10^4/mL and inoculated in T25 culture bottles (3 ml/bottle), which were transferred in the 5% CO_2 incubator at 37 ℃ for 24 h. The culture medium was discarded, to which was added drugs. Three parallel wells were set for each group. Then the cells were incubated in the 5% CO_2 incubator at 37 ℃ for 48 h. Thereafter the cells were washed twice with 1×PBS. The single cell suspension was fixed by 70% ethanol and stored at 4℃, which was washed with PBS to remove the fixing solution prior to staining. Then the cells were incubated in a 37℃ waterbath for 30 min after adding 100 μl of RNase A, and illuminated for 30 min after adding 400 μl of PI. The red fluorescence at 488 nm was recorded.

Statistical analysis

SPSS 19.0 software was used for statistical analysis. The mean statistics were first subjected to the variance homogeneity and normality tests, based on which the data were then subjected to the t test or nonparametric statistics. The inhibitory rates were compared by the Chi-square test.

Results

Cell morphology 24 h after treatment

The normal cells were polygonal, spindle-like, elliptical, normally spread, and densely arranged. The treated cells became spherical, and the cell gaps were enlarged. The numbers of all the treated groups decreased (Figure 1).

Effects of drugs on cell viability

Sorafenib and AGAP

The IC_{50} of sorafenib was calculated as 14.9 μg/ml. The inhibitory effects of sorafenib on SGC7901 cells are shown in Table 1 and Figure 2A. Thereafter, we set the concentration of AGAP at 5 ug/ml based on the above results. The inhibitory effects of AGAP on SGC7901 cells are exhibited in Table 1 and Figure 2B.

Table 1. Effects of sorafenib and AGAP on cell viability

	Sorafenib					AGAP				
Concentration (ug/ml)	100	27	10	8.1	1	30	20	10	5	2.5
Inhibitory rate (%)	96.3	83.5	26.7	23.9	0	32.4	28.7	27.4	26.3	0.1

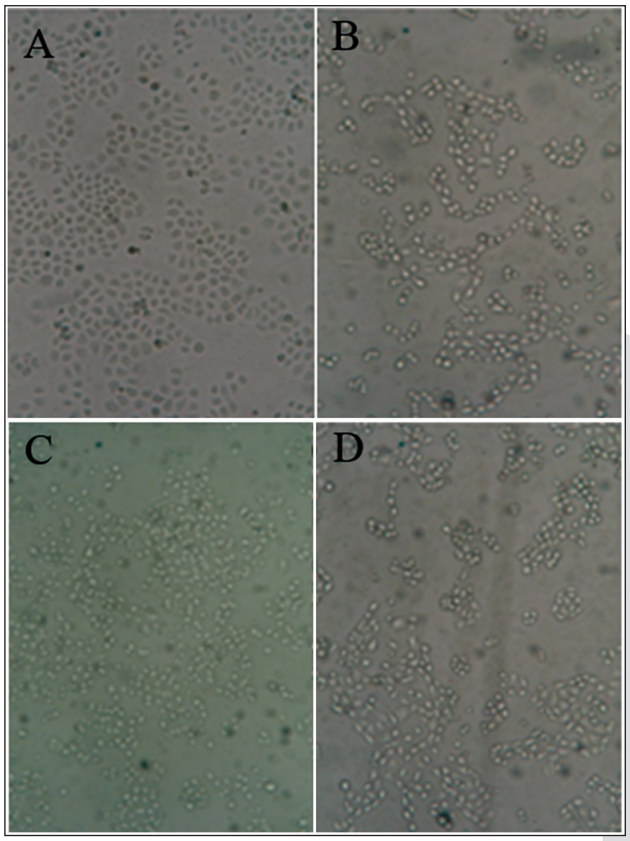


Figure 1. Cell morphology (A: control; B: formula group; C: sorafenib group; D: AGAP group)

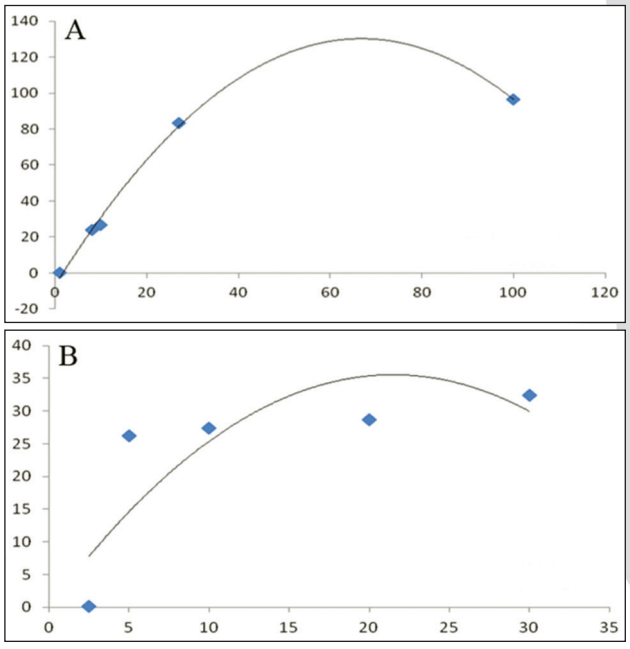


Figure 2. Inhibition curves of sorafenib (A) and AGAP (B)

Spleen-stomach nourishing formula

TCM spleen-stomach nourishing formula at the concentration of 0.01% of the original solution ($C_{original\ solution}$ = 2.6 g/ml) had already inhibited the cell growth significantly (Table 2, Figure 3).

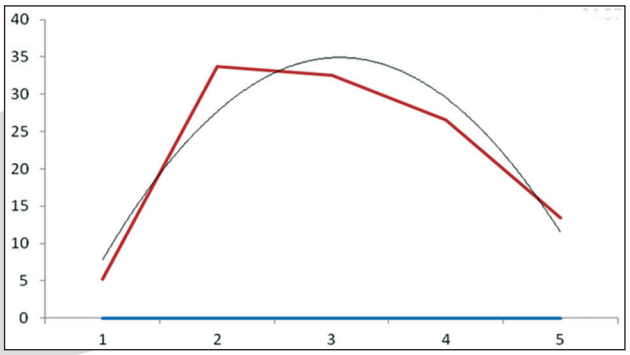


Figure 3. Inhibition curve of the formula

Based on the above results, 15 μg/ml sorafenib, 5 μg/ml AGAP and the formula at the concentration of 0.01% of the original solution were used in the following test.

Effects of drugs on cell invasion

The stained cells in the treatment groups were less than those in the control group (Figure 4). The cell numbers of the treated groups are summarized in Table 3.

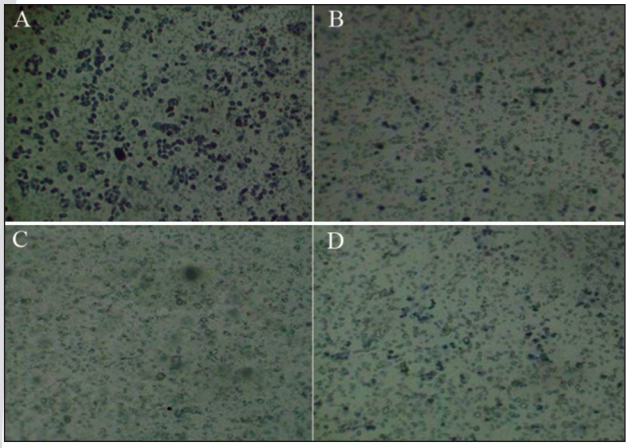


Figure 4. Cell numbers (A: control; B: formula group; C: sorafenib group; D: AGAP group)

Effects of drugs on cell apoptosis

The apoptosis results of the treatment groups and the control group (Figure 5) as well as those

Table 2. Effects of the formula on cell viability

Concentration (ratio to the original solution)	0.01	0.001	0.0001	0.00001	0.000001
Inhibitory rate (%)	5.2	33.72	32.6	26.6	13.4

Table 3. Effects of drugs on cell invasion

	Control	Formula	Sorafenib	AGAP
	187	31	32	78
	272	81	79	245
	250	78	24	67
	273	67	15	55
	204	79	32	66
	176	68	44	45
	246			219
Average	229.7	67.3	37.7	110.7
Standard deviation	40.2	18.7	22.4	83.8
P value (compared to the control)		2.0×10^{-6}	5.2×10^{-7}	0.005

of the spleen-stomach nourishing formula group and the control group were compared (Figure 6), respectively. The apoptotic rates of the two groups are listed in Table 4, in which the early-stage ($P<0.05$) and late-stage ($P<0.01$) apoptotic rates of the treated group are significantly higher than those of the control group.

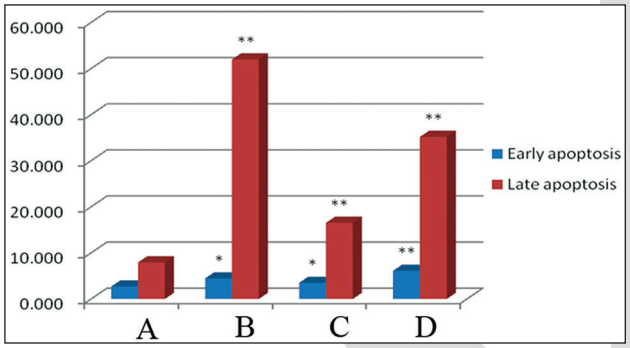
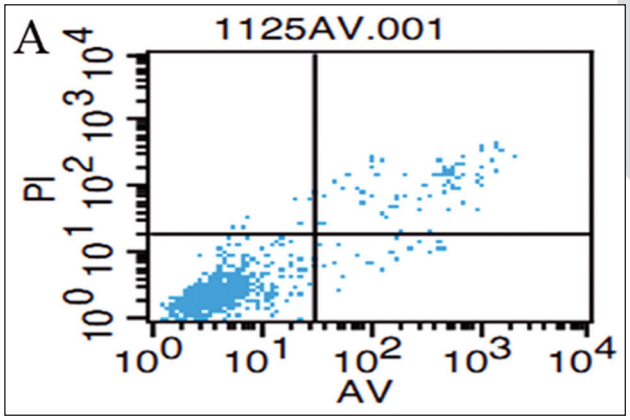


*Figure 5. Cell apoptosis induced by different group (A: control; B: formula group; C: sorafenib group; D: AGAP group) (** p<0.01, * p<0.05)*



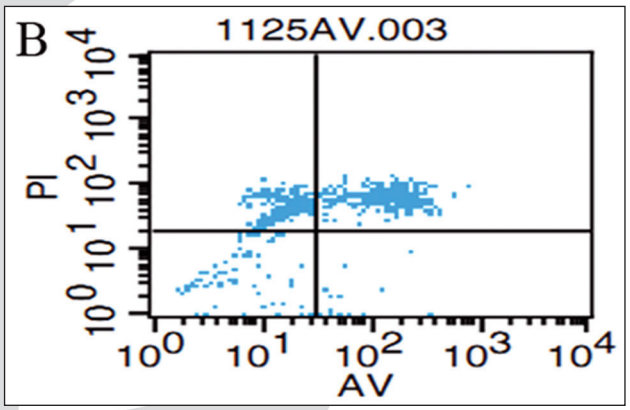


Figure 6. Flow cytometry images of cell apoptosis (A: control; B: formula group)

Table 4. Apoptotic rates (%) of the control and formula groups.

	Early apoptosis	Late apoptosis
Control	2.7033±0.1595*	7.9433±0.3647**
Formula	4.4767±0.1595*	51.9733±2.0899**

**P<0.05; **P<0.01*

Effects of drugs on cell cycle

The effects of all groups on the cell cycle are shown in Figure 7, and the cell cycles of the formula and the control group are compared (Figure 8). Compared to the control group, the spleen-stomach nourishing formula lowered the percentage of the S phase cells, prolonged the duration of G2/M phase cells and did not influence the percentage of G1 phase cells.

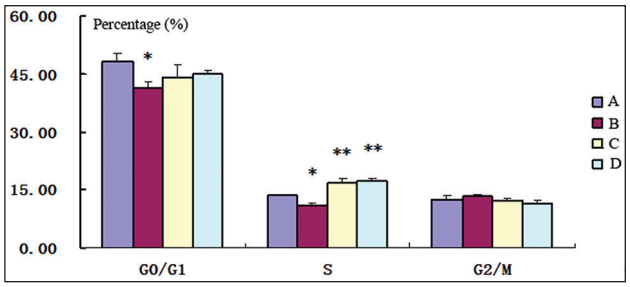
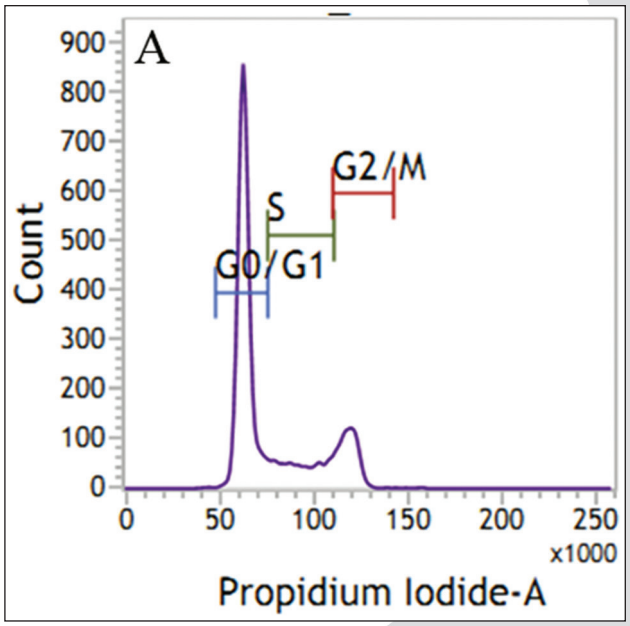


Figure 7. Cell cycles (A: control; B: formula group; C: AGAP group; D: sorafenib group)



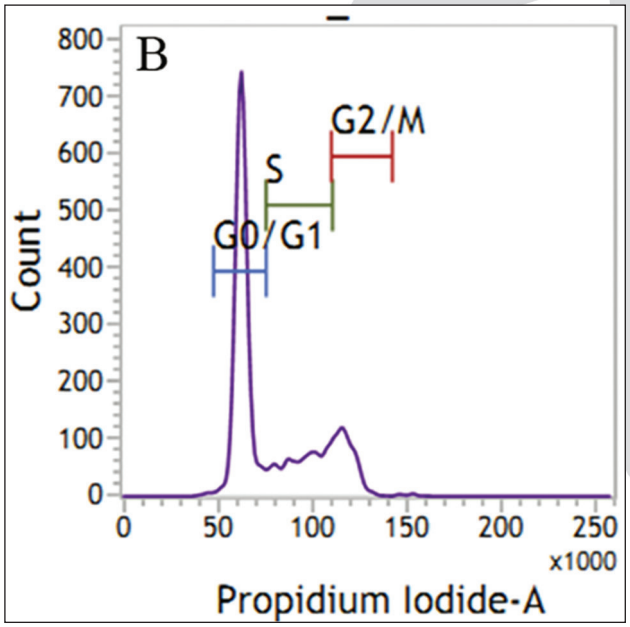


Figure 8. Flow cytometry images of cell cycle (A: control; B: formula group)

Discussion

Currently, gastric cancer is mainly being treated by surgeries and chemotherapy, but the treatment combined with TCM is also feasible (6). TCM fights against tumors via multiple targets and pathways and boosts immunity with rare side effects and drug resistance. TCM controls and eliminates neoplastic foci after surgeries by strengthening spleen, detoxifying and removing blood stasis, aiming to maintain and restore the systematic functions (6,7). The spleen-stomach nourishing formula herein has been applied clinically in the treatment of gastric cancer for several years with satisfactory results. Therefore, we studied the impact of the formula on the treatment of gastric cancer from the molecular biology point of view, which provides evidence for the clinical use.

The MTT test exhibits that the formula triggered the apoptosis of 50% of the cancer cells at low concentrations similar to the conventional antitumor agents did. The Transwell test shows that the number of the formula-treated cells (67.3±18.3) that penetrated the filtration membrane was significantly lower than that of the control group (229.7±40.2) ($P<0.01$), indicating that the formula resisted to cell invasion evidently.

Abnormal cell apoptosis is directly associated with tumors, viroses, degenerative lesions and etc. Cell apoptosis is directly dominated by the genes in cell and indirectly regulated by external factors (e.g. TCM) that affect gene expression and the activation of the resulting products. It has been previously reported that the activation of PI3K-Akt signaling pathway is disturbed in most tumor spectra. This study also supports the conclusion in the significantly higher apoptotic rate of the formula group than that of the control group particularly in the late stage ($P<0.01$). Many pharmacological ingredients in the compound formula may induce the apoptosis of tumor cells, which need to be carefully screened to effectively treat tumors (8).

Moreover, the formula apparently reduced the number of S phase cells and increased that of G2/M phase cells, suggesting that it shorted the S phase as well as hindered the G2/M phase. However, the formula did not inhibit the G0/G1 phase cells. Similarly, Xiang et al. (9) found that quercetin blocked the growth of SGC7901 cells in the G0/G1

phase by inhibiting the early DNA synthesis, but it hindered that of BGC823 cells in the S phase. In other words, variously differentiated tumor cells were inhibited by quercetin in different patterns. Therefore, the influence of the formula on other tumor cells is still in need of further investigation.

Conclusion

In summary, we studied the in vitro effects of the spleen-stomach nourishing formula on the inhibition, invasion, apoptosis and cell cycle phase of gastric cancer cell line SGC7901 by MTT, Transwell and flow cytometry. In addition, we compared the effects of the formula with those of sorafenib and AGAP. The MTT test shows that the inhibitory rates of the formula on the cells were concentration dependent. The Transwell test exhibits that the number of the formula-treated cells penetrating the filtration membrane was significantly lower than that of the control group, indicating that the formula remarkably prevented the cells from invasion. The flow cytometry test reveals that the formula induced more cells to apoptosis and extended the G2/M phase compared to the control group did. The formula displayed excellent in vitro antitumor effects, which may be further applied in vivo and clinically.

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Relationship between fat-free mass and metabolic syndrome in Korean adults: A community-based study

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Abstract

The purpose of this study was to examine whether the percentage of fat-free mass (%FFM) was related to metabolic syndrome (MetS) in a selected sample of Korean adults aged over 20 years (231 adults; men, 55; women, 176); in addition, the subjects of this study were recruited from people who visited a single public health promotion center for a medical checkup in Seoul, Korea between November 1, 2010 and October 30, 2011. We based our definition of MetS on the standard definition in The National Cholesterol Education Program's Adult Treatment Panel III report. Our results indicated that the prevalence of MetS was 30.9% in men and 32.4% in women. The association between %FFM and MetS was assessed using multivariate logistic regression analysis after adjustment for age, sex, sleep duration, mental stress, education level, economic status, frequency of drinking and smoking, and vigorous, moderate, and light physical activity. The odds ratios (ORs) (95% confidence interval [CI]) for the relationship between MetS and %FFM were 1.163 (0.436–3.102, p = 0.763) for low %FFM; 0.345 (0.129–0.986, p = 0.047) for high %FFM; and 0.298 (0.093–0.956, p = 0.042) for very high %FFM, compared with very low %FFM. These results show that high %FFM decreased the prevalence of MetS by 65.5%, and very high %FFM decreased the MetS prevalence by 70.2%, compared to very low %FFM. Thus, we conclude that %FFM was inversely associated with the prevalence of MetS in Korean adults.

Key words: Fat free mass, Lean body mass, Metabolic syndrome, Korean

Introduction

According to the U.S. Department of Health & Human Services, the prevalence of metabolic syndrome (MetS) in adults aged 20 years or more in the US in 2009 was 35.1% for men and 32.6% for women (1). Furthermore, according to the Korea National Health Insurance Corporation, the prevalence of MetS among Korean adults aged over 30 years in 2012 is 31.4% for men and 18.4% for women. Additionally, the National Health Insurance Corporation's report showed that the prevalence of MetS in Korea has been increasing year by year (2). This indicates that MetS is becoming a serious public health problem not only in the US but also in South Korea.

MetS is associated with unhealthy lifestyle-related patterns such as weight gain, decreased physical activity, and an unhealthy diet (3-5). MetS is also associated with an increased risk of type 2 diabetes, cardiovascular disease, and all-cause mortality via abdominal obesity, hypertension, hyperglycemia, and dyslipidemia, which constitute a clustering of metabolic risk factors (6-8). Moreover, MetS is strongly associated with the inflammatory system because of its association with insulin resistance (9).

Previous studies have reported that the inflammation process is linked with muscle strength (10-11). Other studies have shown that MetS is strongly associated with lower levels of muscle strength (12-13). Thus, it can be hypothesized that low muscle strength is a significant risk factor for MetS.

Muscle mass and muscle strength are based on the percentage of fat-free mass (%FFM) of body weight (14). Even though %FFM reflects muscle strength, there is no or little evidence that %FFM

is associated with the prevalence of MetS. Moreover, to the best of our knowledge, there have been no studies on the association between %FFM and MetS in Koreans. Thus, the purpose of this study was to examine whether %FFM was related to MetS in a selected sample of Korean adults.

Methods

Subjects

The subjects of our study were 231 adults (men, 55; women, 176), aged more than 20 years who visited a single public health promotion center in Seoul, Korea between November 1, 2010 and October 30, 2011. Each subject was measured for the following MetS components: waist circumference, high density lipoprotein cholesterol (HDL-C), blood pressure, triglycerides (TG), and fasting blood glucose. All study procedures were approved by the Human Care and Use Committee of the S-gu Community Health Center, and all subjects completed a written consent form before experimental participation in this study. The characteristics of the subjects are shown in Table 1.

Dependent variables

According to The National Cholesterol Education Program's Adult Treatment Panel III (ATP-III), the risk factors for MetS comprise a large waist circumference (≥88 cm for women and ≥102 cm for men), lower HDL-C levels (<50 mg/dL for women and <40 mg/dL for men), higher blood pressure (≥130/80 mmHg), higher TG levels (≥150 mg/dL), and higher fasting blood glucose levels (≥100 mg/dL). For the purposes of this study, we defined subjects with more than 3 MetS risk factors in the ATP-III guideline as MetS individuals. Conversely, subjects with fewer than 2 risk factors according to the guideline were defined as healthy (15).

TG, HDL-C, and glucose concentrations were measured using the ADVIA 1650 automated analyzer (Bayer HealthCare Ltd. Tarrytown, NY, USA). Pureauto S TG-N (Daiichi, Japan), Cholestest N-HDL (Daiichi, Japan), and Hexokinase (Daiichi, Japan) were used to determine TG, HDL, and glucose concentrations, respectively.

After the blood concentrations were assessed, we measured the waist circumference of each subject at the trunk region, midway between the lower costal margin (bottom of the lower rib) and the iliac crest (top of the pelvic bone); this was done while subjects stood with their feet approximately 25–30 cm apart. A tape was carefully fitted around the subjects' trunks without compressing any underlying soft tissues, and the circumference was measured at the end of a normal expiration, to the nearest 0.5 cm (16).

After the subjects had rested for over 10 min in a sitting position, the systolic and diastolic blood pressure were measured at the right brachial artery by a specialist nurse using a mercury sphygmomanometer (Alpk, Japan). Blood pressure was measured thrice with 2-min intervals between each measurement. The specialist nurse then determined the average blood pressure values (17).

Independent variables

We evaluated the %FFM using an 8-polar bioelectrical impedance instrument (InBody-720, Biospace, Seoul, Korea). This instrument uses 8 tactile electrodes to measure the resistance of the arms, legs, and trunk at frequencies of 5, 50, 250, 500, and 1000 kHz. Electrodes are in contact with the palm and thumb of each hand and with the anterior and posterior aspects of the sole of each foot (18). All subjects were prohibited from consuming any food or drink for 4 h, performing any exercise for 12 h, and urinating immediately before the impedance measurement. Further, we recommended the subjects to wear light clothing and remove all metallic items so as not to interrupt the electric current during measurement. All methods used for assessing body composition were based on the recommendations of the book *Applied Body Composition Assessment* (19).

According to the central limit theorem, if the number of subjects in each group is more than 30, the study's data are likely to approximate a normal distribution and be reliable (20). Hence, after %FFM was measured in the 231 study subjects, the data were classified into the following 4 groups: [1] very low %FFM (N = 58), [2] low %FFM (N = 57), [3] high %FFM (N = 58), and [4] very high %FFM (N = 58). The quartile cutoff points for %FFM were determined based on these data.

Covariate variables

The lifestyle-related factors affecting MetS were evaluated for each participant based on the self-reported data in response to 8 questions. We

Table 1. Subject characteristics

Variable		Male (n = 55)	Female (n = 176)	Total (n = 231)
Age (years)		53.44 ± 11.73	53.20 ± 9.85	53.26 ± 10.31
Height (cm)		167.84 ±6.57	156.97 ± 4.90	159.56 ± 7.07
Weight (kg)		70.95 ± 11.61	57.76 ± 7.52	60.85 ± 10.28
Body mass index (kg/m^2)		25.02 ± 3.40	23.48 ± 2.85	23.85 ± 3.05
Prevalence of metabolic syndrome *n* (%)	Yes	17 (30.9)	57 (32.4)	74 (32.0)
	No	38 (69.1)	119 (67.6)	157 (68.0)
Metabolic syndrome components	Waist circumference (cm)	85.56 ± 8.23	77.82 ± 8.86	79.65 ± 9.30
	HDL-C (mg/dl)	45.31 ± 12.20	53.21 ± 12.48	51.33 ± 12.84
	Systolic blood pressure (mmHg)	132.24 ± 17.67	127.06 ± 17.92	128.29 ± 17.96
	Diastolic blood pressure (mmHg)	82.73 ± 12.84	79.62 ± 12.26	80.36 ± 12.44
	Triglyceride (mg/dl)	166.44 ± 98.67	125.05 ± 77.09	134.90 ± 84.37
	Fasting blood glucose (mg/dl)	106.35 ± 27.66	103.36 ± 29.91	104.07 ± 29.35
Frequency of smoking *n* (%)	Non-smoker	38 (69.1)	171 (97.1)	209 (90.5)
	Ex-smoker	11 (20.0)	1 (0.6)	12 (5.2)
	Current smoker	6 (10.9)	4 (2.3)	10 (4.3)
Frequency of drinking *n* (%)	Non-drinker	41 (74.5)	124 (70.5)	165 (71.4)
	Once per month	3 (5.5)	32 (18.2)	35 (15.2)
	2–3 times per month	4 (7.3)	15 (8.5)	19 (8.2)
	Over 4 times per month	7 (12.7)	5 (2.8)	12 (5.2)
Sleep duration *n* (%)	<5 h	5 (9.1)	13 (7.4)	18 (7.8)
	6 h	6 (10.9)	29 (16.5)	35 (15.2)
	7 h	6 (10.9)	28 (15.9)	34 (14.7)
	>8 h	38 (69.1)	106 (60.2)	144 (62.3)
Mental stress *n* (%)	Very low	35 (63.6)	87 (49.4)	122 (52.8)
	Low	3 (5.5)	8 (4.5)	11 (4.8)
	High	11 (20.0)	55 (31.3)	66 (28.6)
	Very high	6 (10.9)	26 (14.8)	32 (13.9)
Education level *n* (%)	Elementary school or lower	3 (5.5)	22 (12.5)	25 (10.8)
	Middle school	5 (9.1)	21 (11.9)	26 (11.3)
	High school	19 (34.5)	71 (40.3)	90 (30.9)
	College or higher	28 (50.9)	62 (35.2)	90 (30.9)
Economic status *n* (%)	Very poor	22 (40.0)	62 (35.2)	84 (36.4)
	Poor	6 (10.9)	33 (18.8)	39 (16.9)
	Rich	25 (45.5)	68 (38.6)	93 (40.3)
	Very rich	2 (3.6)	13 (7.4)	15 (6.5)
Vigorous physical activity	No	37 (67.2)	115 (65.2)	152 (65.8)
	Once per week	7 (12.7)	14 (8.0)	21 (9.1)
	Twice per week	2 (3.6)	14 (8.0)	16 (6.9)
	Thrice per week	3 (5.5)	12 (6.8)	15 (6.5)
	4 times per week	3 (5.5)	11 (6.3)	14 (6.1)
	Over 5 times per week	3 (5.5)	10 (5.7)	13 (5.6)
Moderate physical activity	No	33 (65.9)	109 (62.0)	142 (61.4)
	Once per week	5 (9.1)	15 (8.5)	20 (8.7)
	Twice per week	4 (7.3)	18 (10.2)	22 (9.5)
	Thrice per week	5 (9.1)	15 (8.5)	20 (8.7)
	4 times per week	3 (5.5)	5 (2.8)	8 (3.5)
	Over 5 times per week	5 (9.1)	14 (8.0)	19 (8.2)
Light physical activity	No	31 (56.4)	67 (38.0)	98 (42.5)
	Once per week	5 (9.1)	10 (5.7)	15 (6.5)
	Twice per week	2 (3.6)	11 (6.3)	13 (5.6)
	Thrice per week	5 (9.1)	22 (12.5)	27 (11.7)
	4 times per week	2 (3.6)	8 (4.5)	10 (4.3)
	Over 5 times per week	10 (18.2)	58 (33.0)	68 (29.4)

HDL-C, high density lipoprotein cholesterol; data are presented as mean ± SD or n (%)

also used data from answers to 3 questions in the self-administered International Physical Activity Questionnaire (21). The questions and their responses are given below:

1. Age: the self-reported age of the subjects was used without modification.
2. Sex: the 2 responses were (1) male and (2) female.
3. Sleep duration: the 4 responses were (1) <5 h, (2) 6 h, (3) 7 h, and (4) >8 h.
4. Mental stress: the 4 responses were (1) very low mental stress, (2) low mental stress, (3) high mental stress, and (4) very high mental stress.
5. Education level: the 4 responses were (1) elementary school or lower, (2) middle school, (3) high school, and (4) college or higher.
6. Economic status: the 4 responses were (1) very poor, (2) poor, (3) rich, and (4) very rich.
7. Frequency of drinking: the 4 responses were (1) non-drinker, (2) once per month, (3) 2–3 times per month, and (4) over 4 times per month.
8. Frequency of smoking: the 3 responses were (1) non-smoker, (2) ex-smoker, and (3) current smoker.
9. Vigorous physical activity (PA) per week: the 6 responses were (1) no vigorous PA, (2) once, (3) twice, (4) thrice, (5) 4 times, and (6) over 5 times.
10. Moderate PA per week: the 6 responses were (1) no moderate PA, (2) once, (3) twice, (4) thrice, (5) 4 times, and (6) over 5 times.
11. Light PA per week: the 6 responses were (1) no light PA, (2) once, (3) twice, (4) thrice, (5) 4 times, and (6) over 5 times.

Statistical analysis

All results are presented as mean ± standard deviation. Multivariate logistic regression analyses were conducted to determine whether %FFM was related to MetS after adjustment for age, sex, sleep duration, mental stress, education level, economic status, frequency of drinking and smoking, and vigorous, moderate, and light physical activity. Statistical significance was set at $p < 0.05$, and all analyses were performed using SPSS ver. 18.0 (Chicago, IL, USA).

Results

Multivariate logistic regression analyses

The results of multivariate logistic regression analyses of %FFM for the healthy individual and MetS groups of Koreans are shown in Table 2. The odds ratios (ORs; 95% confidence interval [CI]) for the association between MetS and %FFM were 1.163 (0.436–3.102, p = 0.763) for low %FFM; 0.345 (0.129–0.986, p = 0.047) for high %FFM; and 0.298 (0.093–0.956, p = 0.042) for very high %FFM, compared with very low %FFM. These results show that high %FFM decreased the prevalence of MetS by 65.5%, and that very high %FFM decreased the prevalence of MetS by 70.2%, compared to very low %FFM.

Discussion

The purpose of this study was to examine the association between %FFM and MetS in Korean adults. The results from this study show that MetS might be a risk factor for a decline in muscle mass.

Jurca *et al.* (2005) reported that muscle strength that was determined by bench and leg press and adjusted for weight was inversely associated with

Table 2. The results of multivariate logistic regression analyses of %FFM for the healthy individual and MetS groups of Koreans (n = 231)

	Group	β	SE	OR	95% CI	p
Metabolic syndrome groups (Yes / No)	Very low %FFM	Ref.				
	Low %FFM	0.151	0.501	1.163	0.436-3.102	0.763
	High %FFM	-1.065	0.536	0.345	0.120-0.986	0.047*
	Very %FFM	-1.211	0.595	0.298	0.093-0.956	0.042*

%FFM, percent fat free mass; SE, standard error; OR, odds ratios; CI, confidence interval

**p < 0.05, tested by multivariate logistic regression analysis after adjustment for age, sex, sleep duration, mental stress, education level, economic status, frequency of drinking and smoking, and vigorous, moderate, and light physical activity*

the prevalence of MetS in men (22). Furthermore, Sayer *et al.* (2007) reported that impaired muscle strength determined by grip strength was associated with MetS incidence in both men and women (23). However, to the best of our knowledge, there is no study that compares the muscle mass in MetS and non-MetS individuals.

In our study, the increased prevalence of MetS associated with reduced muscle mass remained significant even after controlling for age, sex, sleep duration, mental stress, education level, economic status, frequency of drinking and smoking, and vigorous, moderate, and light physical activity. This finding suggests that lower muscle mass in MetS cannot be explained solely by lifestyle-related factors or by the levels of physical activity but might instead have a specific pathophysiological pathway.

Skeletal muscle plays an important role in glucose disposal; further, lipid content is inversely associated with insulin sensitivity (24). Therefore, low muscle mass may be a marker for impaired glucose disposal by skeletal muscle; this is closely associated with insulin resistance in the whole body. Conversely, insulin resistance could result in deficits in muscle mass; in addition, insulin resistant glucose-intolerant conditions such as MetS, could have adverse effects on muscle contractile function and force generation (25-26).

This study has several limitations. First, as this was a retrospective cross-sectional study, we could not examine cause-and-effect relationships. However, we were able to examine the relationship between %FFM and MetS. Second, because covariate variables such as lifestyle-related factors were categorized based on the self-reported data, these data might be inaccurate. Third, the subjects of this study were recruited from people attending a single public health center for a medical checkup in Seoul, Korea. Hence, the subjects do not represent the entire Korean adult population. Fourth, the study population for this study (N = 231) was relatively small. Conversely, this study was unique because all subjects were Korean. Moreover, the central limit theorem states that if groups comprise more than 30 subjects, a normal distribution is likely to be approximated by the study data; this in turn makes the data reliable (20). Thus, the fact that the experimental groups in the present study included more than 30 subjects means that our study can be considered reliable.

Conclusion

In conclusion, %FFM was found to be inversely associated with MetS prevalence in Korean adults regardless of age, sex, sleep duration, mental stress, education level, economic status, frequency of drinking and smoking, and vigorous, moderate, and light physical activity.

Fund or Acknowledgements

This research was supported by the Sunmoon University research grants in 2011.

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Abstract

In this paper the instructions for preparing camera ready paper for the Journal are given. The recommended, but not limited text processor is Microsoft Word. Insert an abstract of 50-100 words, giving a brief account of the most relevant aspects of the paper. It is recommended to use up to 5 key words.

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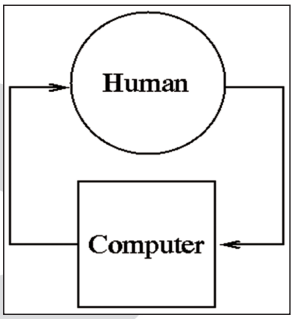

Figure 1. Text here

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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