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Correlation of life quality with clinical and therapeutic characteristics of pediatric patients with TIP 1 diabetes mellitus

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Abstract

Introduction: Diabetes mellitus TYPE 1 (T1DM) is the most common endocrine disorder of childhood with life-long treatment. Therefore, good quality of life is, among better metabolic control and less glucose variability one of the important goals of the therapy.

Aim: to estimate quality of life in pediatric T1DM patients regarding pen and insulin pump treatment, and regarding metabolic control of diabetes and glucose variability.

Patients and Methods: study enrolled T1DM pediatric treated with an insulin pump and multiple pen injections. Pediatric Diabetes Module (PedsQL) 3.0 use for life quality estimation –scale (1-5). Metabolic control was represented by glycated hemoglobin (HbA1c) level and glucose variability by stendard deviation (SD) od mean glycaemia

Results: from 149 T1DM pediatric patients 73 was on insulin pump (M 43/ F 30), 76 were on pen insulin (M46/F30). HbA1c in pump patients was 7,94, and in pen patients 8,61 ($p < 0.05$). Result (1) for life quality was in 1 pump patient, and in 6 pen patients. Result (2) for life quality was in 63% pump patient, and in 65% pen patients and result (4) was in 26 pump patients and in 29 pen patients, No significant difference was between life quality in pump and pen patients ($p = 0,072$), Best regulated patients on both pump and pen had the highest life qulity (4) ($p=0,05$). Ptatients with lower SD of mean glycaemia had higher life quality both for pump and pen treatment. ($p = 0,05$).

Conclusion: The method of administration of insulin (insulin pump / pen) did not have a significant effect on the quality of life of T1DM patients of the pediatric age. Correlation between quality of life and level of metabolic control of pediatric patients with T1DM using IP or pen therapy is at a high level of significance for IP therapy as well as for treated foam. Good quality of life is in a highly significant correlation with lower values of SD medium glycemia.

Key words: diabetes, life quality, insulin treatment, HbA1c, glucose variability

Introduction

Diabetes mellitus TYPE 1 (T1DM) is the most common endocrine disorder of childhood, with an annual incidence of around 3-4% worldwide, and for children up to 7 years and up to 7% (1, 2).

Due to the absolute lack of insulin in patients with T1DM, life-long substitution insulinotherapy is necessary with the adequate, continuous application of other elements of therapy: healthy regulated diet, physical activity, blood glucose control and education (3).

The goal of complete diabetes therapy is to prevent the development and progression of chronic microvascular complications: retinopathy, nephropathy and neuropathy, and chronic macrovascular complications, which prolongs the lifespan with an increase in its quality (4).

Modern therapeutic schemes with insulins of different pharmacokinetics and multiple injections administered by pen should theoretically achieve therapeutic goals. Therapy with the insulin pump significantly addresses the problem of a large number of injections and allows for greater flexibility in the child's meals and activities. However, as many as two thirds of children with T1DM do not have good metabolic regulation of diabetes, nor stable daily glycemia (5).

The ultimate effect of all therapeutic methods is the long-term well-regulated T1DM patient, with minimal glycemic variability, optimal body weight, and effective and simple therapy, which allows

better quality of life. Therefore, the quality of life monitoring (QL) of these patients is an indispensable part of the treatment of diabetes. Good quality of life is, among other things, one of the important goals of the therapy of this disease (6).

The use of the halth-related quality of life (HRQoL) questionnaire with other indicators of the T1DM patient's health status such as: body mass index-BMI, hypoglycemic episodes and glycemic control can help improve our understanding of the problems and possibilities for change (7).

The complex relationship between glycemic control and the quality of life of a pediatric patient with T1DM is very complex. A metabolic well-regulated patient does not always have a good quality of life and vice versa.

The age of the patient, sex, treatment characteristics and HbA1c level do not always give a predictor of the quality of life of T1DM children (8, 9).

Data from the Diabetes Control and Complication Trial (DCCT) study do not confirm the effect of glycemic control or the type of therapeutic regimen on the quality of life of patients (10).

From the standpoint of statistics, the standard deviation of mean glycemia (SD) is the most appropriate parameter of glycemic variability (11). All causes leading to large variations in glycemia are at the same time the causes of hypoglycaemic episodes. A daily limit of variability is needed to help patients reduce the incidence of hypoglycaemia. Frequent hypoglycaemia and high glycemic variability significantly affect the quality of life of pediatric T1DM patients (12),

The quality of life is also evaluated in studies with continuous monitoring of glycemia (CGM), as well as in patients on insulin pump therapy. The capabilities of the CGM and the insulin pump to achieve better flexibility in the daily life of the pediatric T1DM patient are set against its load by devices that have to constantly carry on and enter data on them. The dilemma about the impact of these diagnostic-therapeutic procedures on the quality of life exists, and the result is the consequence of the participation of other factors, which have an impact on the quality of the patient's life (13).

Therefore, it is important to determine the quality of life of pediatric patients with TIP 1 DM with significantly relevant methods and to make a correlation between the quality of life and the degree of metabolic control of the disease and the correlation between quality of life and glycemic variability. Resolving the dilemma about the relationship of the therapeutic modus (pen / insulin pump) and the quality of life of pediatric patients with TIP 1 DM would also contribute to the decision making of a patient preferable to the mode of administration of insulin.

Patients and methods

The study included patients aged up to 18 who had diagnose of T1DM and who are treated with an insulin pump and multiple pen injections at the Pediatric Clinic of the Clinical Center in Sarajevo. There were 149 patients in study which lasted 1 year. The study included patients who were diagnosed with T1DM at least 1 year before the start of the study.

The study did not include patients who changed diabetes therapy during the 6 months prior to the start of the study, and patients who for more than six months did not come to control or were hospitalized at the Clinic. Patients were divided into two groups: a group of patients on insulin pump therapy and a group of patients on pen insulinotherapy. Groups were equal in age and gender.

To assess the degree of metabolic regulation of TIP 1 diabetes, the average level of glycated hemoglobin-HbA1c (at least 3 results) in the year of study was taken. HbA1c was done from venous blood by microcolon method with a reference value of HbA1c <6% for non-diabetic patients (14).

Results of HbA1c were distributed in 5 groups by ranges of 1% (15):

1. HbA1c ≤ 7.0% - perfectly regulated diabetes
2. HbA1c 7.1 - 8.0% - acceptable regulated diabetes
3. HbA1c 8.1 - 9.0% - poorly regulated diabetes
4. HbA1c 9.1-10.0% - very poorly regulated diabetes
5. HbA1c > 10.0% - extremely poorly regulated diabetes

Continuous monitoring of glucose was done for at least 3 consecutive days by the following devices: MiniLink RT (REAL-time) system with an insulin pump or Guardian Real Time; or through a continuous glucose monitor (CGM gold system).

The obtained result of standard deviation of mean glycemia (SD) was used as an indicator of glycemic variability. The ideal SD of meann glycemia should be less than a third of the mean

Table 1. Age and gender of patients within groups with different types of insulin application

	Age (years)			
	Insulin pump		pen	
	73		76	
	girls	boys	girls	boys
	n=30	n=43	n=30	n=46
Rank	11	11	10	11
X	14,666	14,279	14,833	13,543
S	3,133	3,172	2,829	3,494
Sx	0,572	0,483	0,516	0,515
median	15,5	14	15	14
max	18	18	18	18
min	7	7	8	7
p	0,607		0,095	
F test	F=0,266<Fk=3,975		F=2,860<Fk=3,970	

glycemia, however, patients with T1DM hardly achieve such a target SD (16).

Pediatric Diabetes Module (PedsQL) 3.0 which measures, for diabetes, the specific quality of life in relation to the disease was use for life quality estimation PedsQL serves to show quality of life in general, focusing on socio-emotional and physical development and comparing them, both between patients with T1DM and healthy children (17).

Parents were doing a test for patients up to age 12, and for more than 12 years, patients performed the test themselves. In statistical data processing, the "t" test and Exact Fisher probability test were used for two independent samples for N less than 80. The value of p <0.05 would be considered significant. The correlation coefficient r (Pearson's coefficient of correlation) was also used.

To determine relationships between variables sizes we used regression analysis and correlation.

To determine relationships between variable sizes we used regression analysis and correlation. These statistical methods measure the change of one variable caused by changes of the second variable. Regression analysis is just about to determine and measure relationships of this type.

Results

There was no significant difference in the age of patients of different sexes within groups with different modus of insulin dministration.

Table 2. Level of glycated hemoglobin (HbA1c) in patients on insulin pump and pen therapy

	HbA1c %	
	Insulin pump	pen
	n=73	n=76
Rank	6,3	5,9
X	7,949	8,61
S	1,185	1,343
Sx	0,138	0,154
median	7,7	8,45
min	5,8	6
max	12,1	11,9
p	**0,0017**	
F- test	**F=10,114>Fk=3,905**	

Table 2 presents statistically significant difference (p <0.05) in the mean HbA1c level between patients on insulin pump and pen therapy.

Table 3. Distribution of patients on insulin pump and pen therapy according to the HbA1c ranking

	Number of patients	
HbA1c, %	pen	Insulin pump
≤ 7,0	8	13
7,1-8,0	21	31
8,1-9,0	23	18
9,1-10,0	12	6
>10,0	12	5
P	0.18	
F -test	F_1=0.156<F_o=0.371<F_2=6.41	

Table 4. Distribution of results for quality of life in patients on therapy with insulin pump and pen

	quality of life (1-5)		
	2	**3**	**4**
Insulin pump	1	46	26
n=73	1,40%	63,00%	35,60%
pen	6	50	20
n=76	7,90%	65,80%	26,30%

Table 5. Quality of life of patients on insulin pump and pen therapy

	Quality of life	
	Insulin pump	**pen**
	n=73	**n=76**
rang	2	2
X	3,34	3,18
S	0,506	0,558
Sx	0,059	0,064
median	3	3
min	2	2
max	4	4
p	0,072	

Table 6. Distribution of HbA1c levels according to results for quality of life in patients on insulin pump and pen therapy

	HbA1c %	
Quality of life	**Insulin pump**	**pen**
2	9,1	10
3	7,94	8,5
4	7,93	8,48

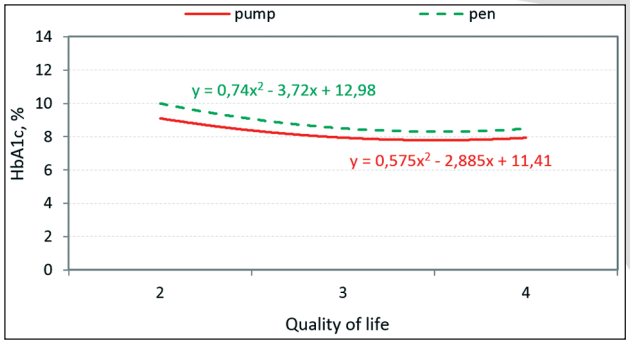

Chart 1. Correlation of HbA1c and quality of life in patients on insulin pump therapy and pen therapy

Table 7. Correlation of HbA1c and quality of life in patients on insulin pump therapy

Insulin pump	Quality of life	HbA1c
Quality of life	1	
HbA1c	-0,869*	1

** Correlation coefficient R = -0.869*
** Correlation is significant at 0.05 level*

Table 8. Correlation of HbA1c and quality of life in patients with pen therapy

Pen	Quality of life	HbA1c
Quality of life	1	
HbA1c	-0,871*	1

** Correlation coefficient R = -0,871*
** Correlation is significant at 0.05 level*

Tables 7 and 8 as well as in Chart 1 show the correlation between the quality of life and HbA1c levels for patients on insulin pump and pen therapy. The lower values of HbA1c correspond to better (higher) quality of life and vice versa. Correlation has a level of significance of 0.05.

Table 9. Distribution of mean glycemic SD (CGM) values according to the level of quality of life for patients on insulin pump and pen therapy

	SD of mean glycemia (CGM)	
Quality of life	**pump**	**pen**
2	3,5	4,667
3	3,139	3,648
4	3,073	3,625

Table 10. Correlation of SD medium glycemic (CGM) and quality of life in patients on insulin pump therapy.

Insulin pump	Quality of life	SD
Quality of life	1	
HbA1c	-0,928*	1

** Correlation coefficient R = -0,928*
** Correlation is significant at 0.05 level*

Table 11. Correlation of SD medium glycemic SDG and quality of life of patients on pen therapy

Pen	Quality of life	SD
Quality of life	1	
HbA1c	-0,875*	1

**Correlation coefficient R = -0.875*
**Correlation is significant at 0.05 level*

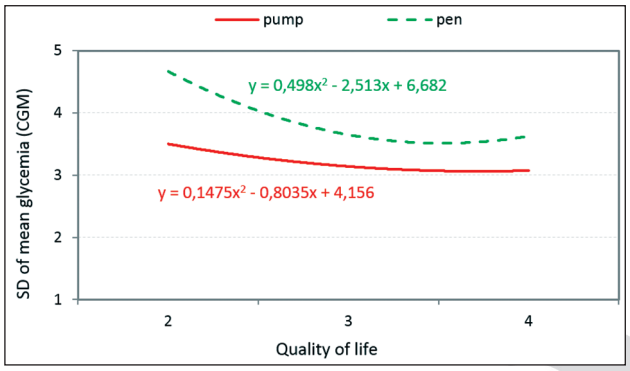

Chart 2. Correlation of SD medium glycemic and quality of life in patients on insulin pump and pen therapy

Tables 10 and 11 as well as Chart 2 show the correlation of SD medium glycemic (CGM) and quality of life of patients on insulin pump and pen. The mean standard deviation is higher in pen treated, but according to the coefficient of correlation, which is very close to 1.0, there is a high significance of this correlation at the level of 0.05. An increase in the value of SD decreases the quality of life and vice versa.

Discussion

A very intensive therapeutic program is the imperative of modern T1DM treatment to reduce the incidence of chronic complications. This treatment is also used in pediatric patients. In addition to better metabolic control of diabetes, intensive therapeutic schemes bring a disorder of communication between the child and parents, as they disturb the usual activities. Behavior is focused on illness, which in every way reduces the quality of life of the whole family, and the quantification of this condition can be done in an appropriate manner. The complex relationship between glycemic control and the quality of life of a pediatric patient with T1DM is very complex. A metabolic well-regulated patient does not always have a good quality of life and vice versa.

The patient's age, sex, treatment characteristics and HbA1c level do not always give a prediction for the quality of life of T1DM children (9).

Data from the DCCT study do not confirm the influence of glycemic control or the type of therapeutic regimen on the quality of patient's life. However, a better metabolic control of the patient reduces stress and family conflict and T1DM child's quality of life with can be better in this case (18).

The quality of life is also evaluated in studies with continuous monitoring of glycemia, as well as in patients on insulin pump therapy. The capabilities of the CGM and the insulin pump to achieve better flexibility in the daily life of the pediatric T1DM patient are set against its load by devices that have to constantly carry on and enter data on them. The dilemma about the impact of these diagnostic-therapeutic procedures on the quality of life exists, and the result is the consequence of the participation of other factors, which have an impact on the quality of the patient's life (12).

Patients with good metabolic regulation of diabetes need not to have a quality life due to numerous daily activities related to the treatment of diabetes and due to numerous insulin injections that they administer with each meal in order to correct glycemia.

In contrast to well-regulated children, there are T1DM patients with high HbA1c values who do not apply recommended treatment activi t ies. Is their quality of life better because they live with less stress or poor regulation of diabetes a dversely affects these children with T1DM. There was a significant difference between the mean value of glycated hemoglobin (HbA1c) in patients on insu1in pump and pen therapy with a high level of significance of p = 0.0017. Glycated hemoglobin is the basic parameter of metabolic control and accor d ing to the results of the DCCT study predictor of chronic complications (13). The mean HbA1c in our study for patients treated with an insulin pump was 7.949%, and for patients with pen therapy 8.81%. The ranking for HbA1c was similar for patients with insulin pump therapy (5.8-12.1%) and for patients with pen therapy (6.0-11.9%), respectively, from the level of well-regulated T1DM (HbA1c < 7%) to very poor regulation of diabetes (HbA1c> 10%).

The metabolic control criterion for pubertal-adolescent-age children according to ADA standards for the care of pediatric patients should be HbA1c <7.0%, i.e. as in adults, however, at this age there is a high preference for hypoglycaemia, so the real goal is that patients can achieve HbA1c <7.5%. The ISPAD criterion for preferred HbA1c in children and adolescents is ≤ 7.0% (3, 16).

The great interest in initiating insulin pump therapy as well as the remarkable increase in this

type of insulin admnistration stimulates the view of improving quality of life. Patients with scores of 3 and 4 life quality have roughly the same levels of HbA1c. Correlation between quality of life and level of metabolic control of pediatric patients with T1 DM in IP and foam therapy is at a high level of significance (p = 0.05) with R = 0.869 for IP therapy and R = 0, 871 for treated foam. The ratio of SD medium glycemia to the quality of life is the same as the relationship of quality of life with HbA1c. Good quality of life is in correlation with lower values of SD medium glycemia, with a high level of significance at 0.05, with R = -928 for treated IP, and with R = -928 for treated IP, and with R = -875 for patients on pen therapy.

In his study, Almzadeh R compares results of metabolic control, parameters of variability and quality of life of preschool children with T1DM. Before and after initiation of IP therapy. There was no significant difference in the level of HbA1c, the frequency of hypoglycaemia, while the glycemic variability expressed by the MAGE value was reduced to IP therapy. There were no changes in the quality of life before and after IP initiation (19).

Müller-Godeffroy E and associates, after transition to IP therapy in pediatric patients, received significantly better quality of life (p <0.001) for all age groups, with minor care and parental intervention related to hypoglycemic episodes (20).

Comparison of the quality of life of patients on pen therapy and insulin pump was performed by Valenzuela JM with associates in patients aged 5-17 years. There was no difference in the quality of life between patients relative to the mode of administration of insulin, nor in relation to other clinical parameters (21).

Greek authors Emmanouilidou E. et al. did not find that poor metabolic control, intensity of treatment, and age and increased BMI affect the quality of life of children with T1DM age 2-18 years (22).

A study by Wagner VM and associates, and Laffel LM research, found better quality of life for T1DM children aged 8-16 years in metabolically regulated patients who are on intensive and insulinotherapy and younger age (6, 23).

The results of Ausilia E and associates confirm that a higher number of glycemic insufficiency daily, and a better metabolic control of diabetes with the younger age of the child and the earlier onset of diabetes results in a better quality of life of T1DM pediatric patients (7).

The positive effect of good metabolic control on the quality of life of T1DM pediatric patients was also established by Hanberger L (24).

According to Kuwait study for estimation of QoL, the use of multiple daily injections was associated with worse QoL in the younger age group (2-4 years, p <0.05). However, in the other 3 age groups, the use of more than 2 injections was associated with significantly higher scores, according to both child and parent reports (p <0.001). Furthermore, the use of a continuous insulin infusion pump was associated with better QoL in all 4 age groups. There was a significant correlation between total QoL score and mean HbA1c (r2 = -0.7, p = 0.001). In all age groups, subjects with better metabolic control had better total (Fig. 3), emotional (r2 = -0.4, p <0.05) and social (r2 = 0.043, p <0.05) QoL scores (25).

There was a significant relationship between insulin regimen and QoL in all age groups. Patients using insulin pumps, even those less than 4 years old, had better QoL compared to their counterparts taking multiple daily insulin injections as previously reported. Association between HbA1c and QoL is so consistent that it is now justified to consider QoL and metabolic control equally important in the management of T1DM Whether good metabolic control increases QoL or high QoL enhances metabolic control, or both, is a field that requires further study (26, 27, 28).

The PedsQL scores were also higher (better) for those using an insulin pump compared with those participants who injected insulin, with an HbA_{1c} level of less than 9%, no comorbid conditions (29).

Conclusion

The method of administration of insulin (insulin pump / pen) did not have a significant effect on the quality of life of T1DM patients of the pediatric age. Correlation between quality of life and level of metabolic control of pediatric patients with T1DM using IP or pen therapy is at a high level of significance for IP therapy as well as for treated foam. Good quality of life is in a highly significant correlation with lower values of SD medium glycemia. All available agents and procedures must improve the metabolic control of TIP 1

diabetes and reduce glycemic variability in pediatric patients so that the long-term quality of life of the child and family is at a higher level.

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Prospective single-center observational study of a new dietary supplement containing collagens type I, II, V, and X

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Abstract

Introduction: The present prospective single-center observational study investigated the safety and efficacy of TendoGuard, a new dietary supplement containing collagen type I, II, V, and X.

Methods and materials: A goniometer was used to measure the range of motion, a pain scale (Borg) was applied to subjectively percept the pain, and a properly calibrated sphygmomanometer was utilized to evaluate muscle strength.

Results: The results indicated that administration of 750 mg/day of TendoGuard for 60 days improved essential symptoms in individuals suffering from joint diseases, including range of motion, general pain, and muscle strength. No adverse effects were detected during the observation period.

Conclusion: The results support the view that TendoGuard may be administered to patients suffering from joint diseases. These data encourage its use for patients suffering from degenerative joint diseases, including cartilage injuries, connective tissue disorders, polychondritis, joint defects, osteoarthritis, and rheumatoid arthritis.

Key words: Collagen, Joint Diseases, Range of Motion, General Pain, Muscle Strength.

Introduction

TendoGuard, an association of collagens type I, II, V, and X, is a dietary supplement that may be beneficial for patients suffering from degenerative joint diseases, including cartilage injuries, connective tissue disorders, polychondritis, joint defects, osteoarthritis, and rheumatoid arthritis. Its use in the treatment of degenerative joint diseases has increasingly gained support in medical community, and among consumers[(1)].

It has been verified, in preclinical studies, that orally administered collagen is thoroughly absorbed by the intestine and circulated in the blood stream, remaining in the gastrointestinal tract. It was also revealed that a significant amount of collagen hydrolysate-derived peptides reaches cartilage tissue[(2)]. Additionally, it was exposed that treatment of cultured chondrocytes induced a statistically significant dose-dependent increase in collagen synthesis of the chondrocytes in cell culture experiments[(3)].

Hyaline articular cartilage is a highly specialized avascular tissue that covers the surface of the diarthrodial joints, consisting of 5% of cells, the chondrocytes, which are immersed in the extracellular matrix. These cells present in small proportions are considered the metabolic center and producer of the vast extracellular matrix found in cartilage, composed basically of water, proteoglycans, collagen and other proteins. Water accounts for about 65-85% of the dry weight of the fabric, while the major macromolecules, such as collagen and proteoglycans, account for about 10% to 30% of the dry weight of the fabric, respectively[(4)].

The composition and complex structural organization between collagen and proteoglycans ensures the inherent properties of articular cartilage, such as strength, elasticity and compressibility, necessary to dissipate and cushion the forces, as well as reduce friction, to which the diarthrodial joints are subjected, without much energy expenditure. Therefore, the integrity of the articular cartilage components is essential to ensure normal joint function[(5)].

Collagen is the main structural element that confers tissue resistance; it is known that in addi-

tion to the support function, it participates in cell differentiation, adhesion, migration and proliferation, also exerting antigenic activity[5,6].

The articular cartilage is composed primarily of collagen type II , with at least ten additional collagens, including types III, VI, IX, X, XI and XIII, present as minor constituents[7, 8, 9]. Of these, types II, VI, IX and XI were identified in cartilage in amounts sufficient to be isolated from tissue or from chondrocyte culture[10].

Type V collagen is proportionally the smallest mass component in tissues but plays a key role in tissue proliferation and repair processes. Its presence in the basement membrane of vessels and in some mesenchymal tissues is of extreme importance in the connection between collagen IV of the basement membrane and the loose connective organ, actively participating in the interaction of extracellular matrix components and establishing association with other types collagen[11].

Except for collagen type X, these collagens are also found in structures similar to cartilage, such as vitreous humor of the eye, developing cornea, nucleus pulposus discus and intra-articular meniscus. Recent studies have shown that the organization of collagen molecules in the vitreous and cartilage fibrils is identical[12].

Collagen type X, also cartilage-specific, is a homotrimer, considerably shorter than type II and XI collagens. Type X has helical and non-helical domains, in addition to a large non-helical carboxy-terminal domain. This collagen is more abundant in hypertrophic cartilage, in the transition between cartilage and bone[13].

Based on the findings that collagen is absorbed in its molecular form, accumulating in cartilage, and is able to stimulate chondrocyte metabolism[14], it might be reasonable to use the association of collagens type I, II, V, and X, as a nutritional supplement to activate collagen biosynthesis in chondrocytes in humans, especially patients suffering from degenerative joint diseases. Thus, the aim of this single-center investigation is to extend these earlier findings with TendoGuard.

Methods and materials

In accordance with the ethical standards of the Ethics Committee of Mortec Scientific, Inc. (Cambridge, ON, Canada) on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000 and 2008, this prospective single-center clinical observational study was approved by its responsible committee, and managed in its Department of Clinical Medicine. According to study schedule, the consent form was discussed, signed, and a complete physical examination was executed at screening. Activity level, diet history, medication/supplement use and medical history were recorded.

Subjects' complaints of joint discomfort were recorded using pre- and post-treatment questionnaires to evidence personal data and issues related to an individual's functional quality. A goniometer was used to measure the range of motion[15], a pain scale (Borg) was applied to subjectively percept the pain[16], and a properly calibrated sphygmomanometer was utilized to evaluate muscle strength[17].

Urine was collected for a pregnancy test for women of childbearing potential. A blood sample was taken for determination of alanine transaminase (ALT), aspartate transaminase (AST), bilirubin, blood urea nitrogen (BUN) and creatinine. Upon review of blood test results, eligible subjects were instructed to get an X-ray of the affected joints to confirm diagnosis. A total of 20 subjects were recruited using the inclusion and exclusion criteria outlined in Table 1.

At the first visit, selected subjects, properly informed by the Consent Term approved by the Scientific Committee of the Institute, were assigned to receive 750 mg of TendoGuard™ (Certified Nutraceuticals, Inc., San Diego, CA) daily. At the final visit, subjects were required to come to the clinical division for clinical assessment. A subject treatment diary was completed by each patient throughout the study period to determine product compliance, side effects, and supplementation use.

By GraphPad InStat 3.1, the Wilcoxon's test was used to compare non-parametric variables, whereas the variance analysis (ANOVA) test was used for parametric ones. A significance level of 5% was adopted in all comparisons and statistically significant results were marked with an asterisk (*).

Table 1. Inclusion and exclusion criteria

Inclusion criteria
Males and females 45-75 years old
Females of childbearing potential must agree to use a medically approved form of birth control and have a negative urine pregnant test result
Disorder of the knee for more than three months
Able to walk
Availability for duration of study
Subject agrees not to start any new therapies during the course of the study
Able to give informed consent
Exclusion criteria
History of asthma, history of diabetes
Hyperuricemia
Hypersensitivity to NSAIDs
Abnormal liver or kidney function tests
Abnormal findings on complete blood count
Uncontrolled hypertension
History of allergic reaction to any ingredients in the test product
Hyperkalemia (potassium > 6.2 mmol/L)
History of cancer as well as gastrointestinal, renal, hepatic, cardiovascular, hematological, or neurological disorders
Anticipated problems with product consumption
High alcohol intake (>2 standard drinks per day)
History of psychiatric disorder that may impair the ability of subjects to provide written informed consent
Use of concomitant prohibited medication (narcotics, NSAIDs)
Any other condition that, in the opinion of the investigator, would adversely affect the subject's ability to complete the study or its measures

Results

Baseline characteristics of patients are summarized in Table 2. Where applicable, values are expressed as mean ± standard deviation.

Table 2. Baseline characteristics of patients.

Characteristics of patients	Values
Age (years)	55.9 ± 7.91
Sex (male/female)	10/10
Height (cm)	168.1 ± 8.52
Weight (kg)	81.3 ± 14.6
Systolic blood pressure (mm)	120.5 ± 7.84
Diastolic blood pressure (mm)	80.6 ± 8.33
Heart rate (bpm)	68.9 ± 7.42

The results are presented in Table 3 and Table 4 listing values for average, and standard deviation for each analyzed variable. Statistically significant results are marked with an asterisk (*)

Table 4. Pre- and post- treatment groups.

Comparison	P
Range of motion	0,011*
General pain	0,001*
Muscle strength	0,004*

These results indicate that administration of 750 mg/day of TendoGuard for 60 days improved essential symptoms in individuals suffering from joint diseases, including range of motion, general pain,

Table 3. Range of motion, pain and muscle strength.

	Range of motion[15]		General pain[16]		Muscle strength[17]	
Treatment	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Average	105,22	172,53	8,73	1,92	58,43	104,97
Standard deviation	13,46	10,81	10,54	12,73	10,54	11,73
Standard error	4,22	4,93	4,76	5,48	4,76	5,68

and muscle strength. No adverse effects occurred during the 60-day observation period. The treatment was reported to be well tolerated by subjects.

Discussion and conclusion

Several nutritional supplements, including chondroitin, glucosamine, soybean unsaponifiables and diacerein have emerged as new treatment options for joint disorders in the last few years[18]. The aim of this prospective single-center investigation is to evaluate the safety and the efficacy of a new dietary supplement containing collagens type I, II, V, and X, TendoGuard, which is a complex structural protein that may provide strength and flexibility to connective tissues.

It was investigated, in an observational study, the use of collagen hydrolysate as a nutritional supplement to reduce symptoms of joint damage, with the expectation that this change would reflect improvements in joint health. Individuals were recruited who had not been diagnosed with degenerative joint disease but who complained about joint pain that both the treating physician and the subjects interpreted as being a result of stressful exercising. It was reported that 78% of individuals at the end of the study noticed substantial improvement of their joint symptoms, including range of motion, pain, and muscle strength[19].

The evaluation of muscle strength is an important technique to diagnose the etiology of the disease, and to define rehabilitation strategies. The muscle weakness, which was observed in our study during the pre-treatment assessments, is directly associated with knee joint pain and joint disability[20].

Osteoarthritis results in changes that affect not only intracapsular tissue, as well as periarticular tissues, such as ligaments, capsules, tendons and muscles. Osteoarthritis patients compared to healthy individuals of the same age had muscle weakness, reduced knee proprioception, reduced balance and position sense[21].

The presence of joint effusion, even in small amounts, is a potent inhibitory mechanism reflex muscular activity of the joints. A reduced reflex muscular activity causes hypotrophy and weakness early, with the resultant associated mechanical damages, such as decreased range of motion[22].

Muscle strength declines rapidly during the detention of a member by decreasing the size of the muscle and stress per unit of the muscle cross-sectional area. The largest absolute loss of muscle mass occurs at the beginning of hypotrophy process. The pain inhibits reflex muscular activity, causing atrophy, and muscle weakness[23].

The purpose of this study was to define whether administration of 750 mg of TendoGuard daily would reduce joint pain in patients suffering from joint diseases. The design of the observational study was appropriate to reveal that collagens type I, II, V, and X as a nutritional supplement ingested over 60 days was safe and efficacious in reducing symptoms of joint discomfort. The results of the study provide data supporting the view that TendoGuard may be administered to patients suffering from joint diseases. Further research will elucidate additional benefits from this association of collagens type I, II, V, and X.

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New method of age assessment based on pubic symphysis at skeletal remains

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Abstract

Introduction: Determining the age of human skeletal bones at death requires the analysis of morphological features of the entire skeleton. Age related morphological changes at the public symphysis face are one of the safest and most often used indicators in determining the age of a person in time of death. In the process of court-anthropological assessment and identification of human skeletal remains of unknown identity, determining the age at death, in addition to determining gender and height at death, is one of the most important issues to be answered as precisely as possible. Currently, several standardized methods of pubic symphysis are used when estimating the age at death. The first, more comprehensive study on age conditioned morphological changes was made by the anatomist Todd in 1920. He defined the morphological changes on public symphysis at death through 10 phases, each having a defined range of age. Suchey-Katz in 1986 and Suchey-Brooks in 1990 established the method for pubic symphysis comprising six phases, which was tested on the population of Bosnia and Herzegovina.

The aim of this study was to develop a new method for estimating the age at death of human skeletal remains of men, based on age conditioned morphological changes of pubic symphysis at the population of Bosnia and Herzegovina.

Material and methods: The sample comprised 610 pairs of male pubic symphysis, known year of birth and year of death. The identity was determined by DNA analysis. The youngest person was aged 15, and the oldest 78. Twelve morphological features of face of the pubic symphysis were analysed in total (horizontal ridges and furrows, public tubercle ridges, dorsal margin, ventral margin, rim of pubic symphysis face, general changes at surface of the pubic symphysis face, tendon and ligamentous ossification processes, valves at dorsal and ventral margin, porosity and wearing off of the public symphysis face and ventral rampart. Nine phases of age conditioned morphological changes of pubic symphysis were defined for male BH population.

Results: Methodologically, descriptive statistic was used, regression-correlation analysis and multiple regression model. By using the descriptive statistics, the average age of the analysed sample is 38.33 years, with standard deviation 15,629 years, variation coefficient of 40,775 years, and variation range of 63 years. 50% of units from the sample were aged at death 36 or less while 50% were aged at death over 36. The most frequent units in the sample were those aged 23 at death. Correlation coefficient between actual age at death and the phase related to certain morphological features of pubic symphysis, as well as the phases of certain morphological features are mutually significant **(**$P \leq 0.05$). Statistically significant difference was proved between median values for age at death variable between different consecutive phases for all 12 morphological features of pubic symphysis (Mann-Whitney nonparametric U test). The actual age at death was determined per phases for each of twelve analysed morphological features (95% CI). By unifying the results and considering the crossing per morphological features, new method has been obtained comprising 9 phases, based on age conditioned changes of pubic symphysis. The intervals were determined, in fact the range for each of nine phases of pubic symphysis, average age with standard deviation.

Conclusion: All twelve defined morphological features of pubic symphysis and related phases analysed in this study at the sample of 610 pubic symphyses significantly correlate with aging process. Defining the phases and age conditioned morphological features of pubic symphysis specific for BH male population enabled the develop-

ment of the method for assessment of age at death of skeletal remains.

Key words: Pubic symphysis, age assessment, forensic anthropology, court medicine.

1. Introduction

During the court-anthropology processing and identification of skeletal remains of unknown identity, determining their lifetime age in fact the age at death, in addition to determining gender and height, is one of the most important issues to be answered as precisely as possible.

During the lifetime, the entire human skeleton goes through morphological-structural changes that were the subject to numerous studies, resulting with development of numerous methods for assessment of age at death of human skeleton.

Morphological changes on face of the pubic symphysis are one of the most reliable criteria that consistently follow the aging process. The first standards in the assessment of age at death based on morphological features of pubic symphysis were set by the American anatomist Todd (1920, 1921). He defined morphological changes on pubic symphysis at death through 10 phases, each phase having a defined range of years of age. (1,2). Acsadi and Nemeskeri in 1970 presented the morphological changes on pubic symphysis in five phases, with certain range of years (3). Other authors developed different modifications of Todd's method (4,5). Sucheyeva and Brooksova in 1986 and 1990 developed pubic symphysis method on a large number of samples N=739. (6,7). They presented morphological changes on bones of public symphyses through six phases, shown at the photos and plaster moulds, each phase having a certain range of years.

In a long court-anthropology processing of numerous skeletal remains (over 15000), from the previous BH war, the assessment of their age at death utilizing Todd and Suchey-Brooks method did not give sufficiently precise results, in fact there were some cases of underestimated but also overestimated age at death. Population-specific studies for BiH indicated the lack of original method, in terms of its applicability to other population, as well as very wide ranges of age assessment for certain phases (8, 9). Therefore, the aim of this study was to define the age-conditioned morphological features of pubic symphysis, as well as to develop the method for age at death assessment of skeletal remains, based on age-conditioned morphological changes of pubic symphysis specific for male BH population.

2. Material and methods

The sample for this assessment comprised the pairs of pubic symphysis of groin pelvic bones of 610 men who disappeared during the previous war, aged between 15 and 78. The identity of persons was confirmed by DNA analysis.

Planned assessment included primarily the selection and preparing of pairs of whole and intact pubic symphyses. Afterwards, 12 morphological features of pubic symphysis were defined, which were analysed through aging process, as well as the phases of morphological changes development for each of 12 features ((M1- horizontal ridges and furrows on face of the pubic symphysis with 5 development phases in total, M2- ridges on pubic tubercle with 3 development phases in total, M3-dorsal margin with 4 development phases in total, M4 - ventral margin with 4 development phases in total, M5- frame of the pubic symphysis face with 4 development phases in total, M6-macroscopic changes on the face of pubic symphysis with 4 development phases in total, M7- upper extremity with 4 development phases in total, M8 – lower extremity with 6 development phases in total, M9 - tendon and ligamentous ossification processes with 3 development phases in total, M10 – valves on dorsal and ventral margin with 4 development phases in total, M11 – porous and worn off face and pubic symphysis rim with 4 development phases in total and M12 – ventral rampart with 4 development phases in total).

Finally, total phases were defined based on statistically presented age changes for each morphological change. Descriptive statistics of regression-correlation analysis and multiple regression model were prepared for the analysis.

3. Results and discussion

It has been determined that the average age for the whole tested sample totals 38.33 years, with standard deviation of 15.629 years. Half of the cases (50%) from the sample was aged 36 or less, and the other half (50%) was over 36 years of age at

death. The cases with actual age at death from 20 to 40 dominate in the sample, while there are the least of the persons aged 70 and more. There were the most of the persons aged 23 at death (Table 1).

Table 1. Descriptive statistics indicators for actual age at death variable

Variable: Actual age	
Average	38,33
Median	36
Mode	23
Standard deviation	15,629
Variation coefficient	40,775
Variation range	63
Minimum	15
Maximum	78
Standard average estimation error	0,633
Confidence interval for the average (95% CI)	37,083 - 39,569
Number of observations (*N*)	610
Kolmogorov-Smirnov test (KS test)	$z = 2,505$ $p = 0,000 < 0,05$

Analysis of the correlation between the actual age at death and 12 morphological features with associated phases (Spearman correlation coefficient) showed that all the correlation coefficients were monitored with P value below 0.05, which indicates that all analysed morphological features with their phases significantly correlate with age.

Analysis of difference significance between certain phases of certain morphological features showed that each phase with defined morphological features of pubic symphysis significantly correlates with age changes (from M1 to M12, $p<0.05$).

Statistically significant difference between median values for the variable actual age at death between different consecutive phases was proven for all 12 morphological features of pubic symphysis (Mann-Whitney nonparametric U test).

Actual age per phases was determined for each of twelve analysed morphological features (95% CI) (Table 2).

Table 2. Actual age per phases for analysed morphological features (95% CI)

95% CI for actual age	Phase of observed morphological feature				
	1	2	3	4	5
M1	18	21-22	25-28	32-36	47-49
M2	18-19	21-23	43-45		
M3	18-19	25-27	41-43	57-60	
M4	19-20	26-29	38-40	53-56	
M5	19-20	26-29	40-42	57-60	
M6	18-19	23-25	38-41	53-56	
M7	19-20	25-27	39-41	57-60	
M8	19-20	26-28	40-43	57-61	
M9	26-27	39-41	55-57		
M10	22-23	34-36	40-45	53-56	
M11	20-22	33-36	38-42	54-56	
M12	16-17	19-20	23-24	45-47	

Table 3. Descriptive statistics of newly proposed intervals

Newly proposed interval	Interval	average±standard deviation
1	15-18	16,4±1,0
2	17-25	20,9±2,4
3	19-28	22,7±2,9
4	21-37	28,4±5,3
5	22-45	32,7±7,1
6	25-50	37,0±7,2
7	37-60	48,4±7,0
8	40-70	52,4±8,2
9	50-80	58,9±6,5

Table 4. Results of U test for determining significance of differences between the proposed intervals

Statistics		Mann-Whitney U	Wilcoxon W	Z	p vaule
Intervals	1-2	137	515	-7,765	0,000
	2-3	7535,5	19163,5	-5,128	0,000
	3-4	6760	18085	-10,163	0,000
	4-5	22472	49733	-6,990	0,000
	5-6	29040,5	73591,5	-6,610	0,000
	6-7	9940,5	50695,5	-14,139	0,000
	7-8	23112,5	52758,5	-5,387	0,000
	8-9	12800	47516	-7,522	0,000

Unification of the results and analysis of the crossings per morphological features led to a new method comprising 9 phases based on age conditioned morphological changes of pubic symphysis. The intervals, in fact range of years for each of nine phases of pubic symphysis were determined, average age with standard deviation for each phase (Table 3).

It is evident that the average age has ascending ratio and that there is a significant difference of median values for the variable actual age at death between different consecutive intervals ($p<0.05$), (Table 4).

Model of nine phases

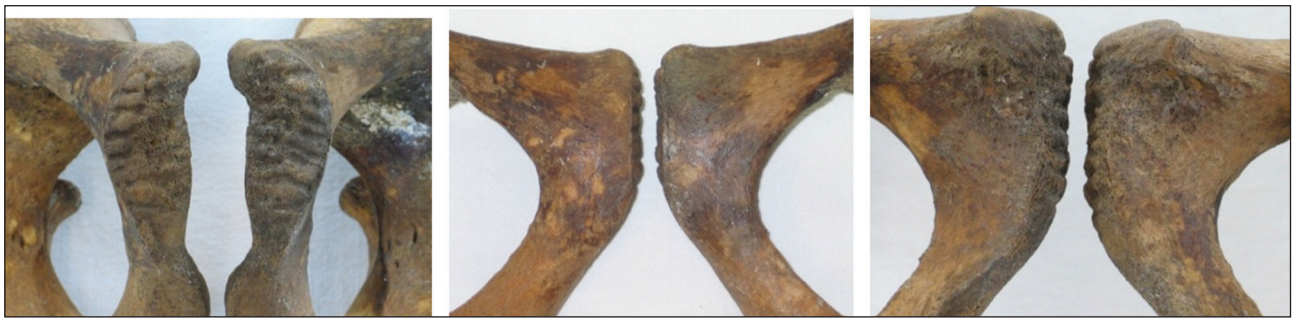
Phase 1 (age 15 - 18)

Symphysal face is convex, the entire surface is wavy, crossed horizontally with bone ridges and furrows extending along the symphyses face width. Ridges are highly elevated and mildly curved in the region of dorsal and ventral rim. Furrows between the ridges are deep and they follow the ridge curve in the region of dorsal and ventral rim. Ridges and furrows on pubic tubercle are clearly expressed and extend along the surface. No signs of forming the dorsal and ventral margin, or signs of bone frame around the face of the pubic symphysis. No visible signs of forming the upper and lower extremities or signs of tendon and ligamentous ossification processes. No signs of forming the bone valves on dorsal and ventral margin, or visible signs of forming the ventral rampart (Pictures 1a, 1b and 1c).

Phase 2 (age 17 - 25)

Face of the pubic symphysis is less convex, horizontal ridges become lower and furrows between them are shallower, filled in along the dorsal rim with new bone of fine texture, more expressed in the bottom part. Ridges on pubic tubercle are still noticed, with signs of their disappearing in the upper part towards ramus. No signs of forming the dorsal margin or it is noticed more towards the lower extremity. Still no visible signs of ventral margin forming. In minor part, there are visible signs of development of the frame of pubic symphysis face on dorsal side. No signs of forming the upper extremity with possible initial limitation of the lower extremity. No signs of tendon and ligamentous ossification processes or valves on dorsal and ventral margin. Signs of forming the ventral rampage are seen (Pictures 2a, 2b and 2c).

Picture 1a. Face *Picture 1b. Back side* *Picture 1c. Front side*



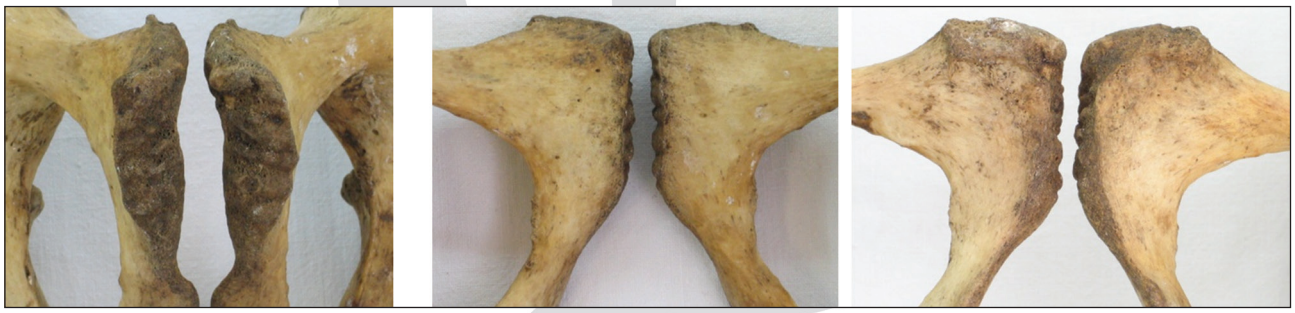
Picture 2a. Face *Picture 2b. Back side* *Picture 2c. Front side*

Phase 3 (age 19 – 28)

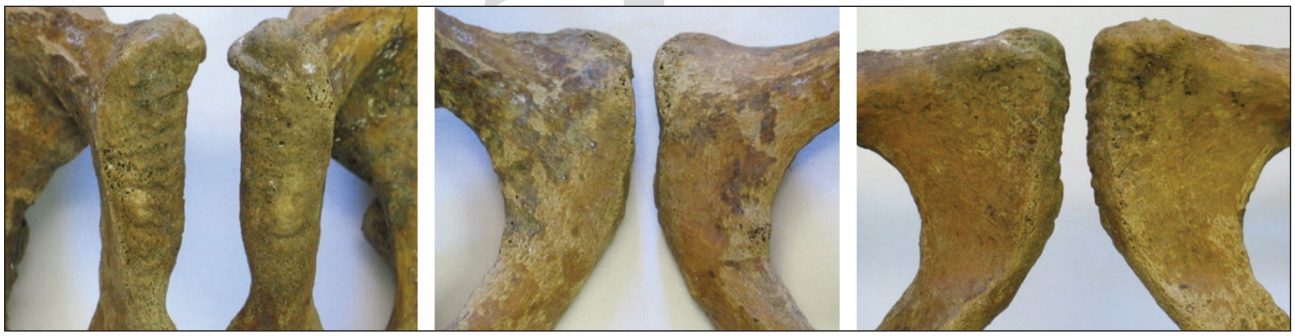
Symphysal face generally with lower and flat horizontal ridges and shallower furrows, particularly towards dorsal rim where the furrows are disappearing, filled with a new bone, with signs of upper tubercle formation. Ridges on pubic tubercle are hardly noticed or they disappeared completely. Dorsal margin properly bordered completely. In minor, upper or lower part, there are visible signs of forming the front wall, as bone extension of upper and lower extremity. Partially formed frame around symphysis face, with initial signs of forming and limiting the upper extremity. Separation of the lower extremity expressed more and better. Still no signs of tendon and ligamentous ossification processes or formation of valves on back and front margin. Ventral rampage emphasized (Pictures 3a, 3b and 3c).

Phase 4 (age 21 – 37)

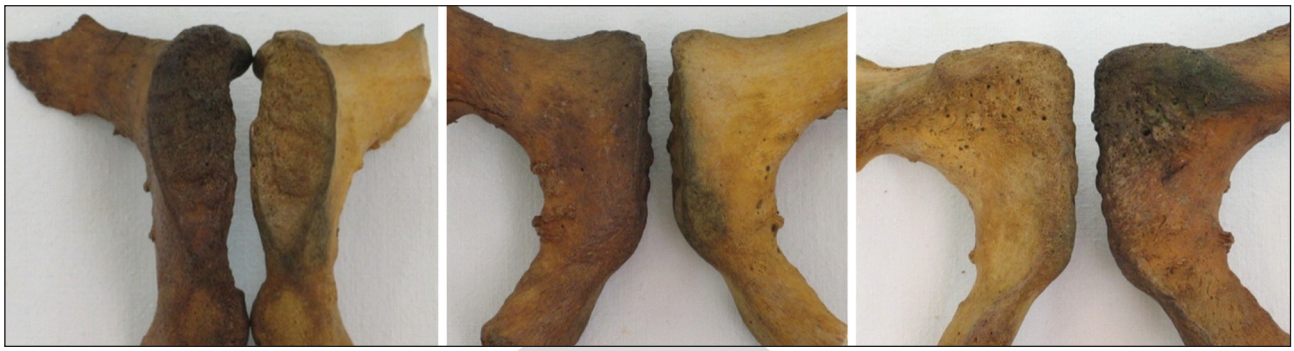
Symphysal face is almost flat, showing generally fine granulation, hardly emphasized low and blunt ridges, which almost disappeared completely, and shallow furrows are filled with new bone. Face is without depression and without signs of porosity, not worn-off, with numerous holes on surface. Ridges on pubic tubercle are missing. Dorsal margin bordered with signs of plateau development and border thickening extending towards the upper segment of dorsal margin. Front wall better, but still not entirely developed. Frame of the symphysis face is still not completely closed. Upper extremity clearly and better defined, but lower extremity separated well from the lower branch of groin bone. There might be the first signs of tendon and ligamentous ossification processes with hardly emphasized valves on dorsal margin. Ventral rampage is disappearing (Pictures 4a, 4b and 4c).



Picture 3a. Face *Picture 3b. Back side* *Picture 3c. Front side*



Picture 4a. Face *Picture 4b. Back side* *Picture 4c. Front side*



Picture 5a. Face *Picture 5b. Back side* *Picture 5c. Front side*

Phase 5 (age 22 – 45)

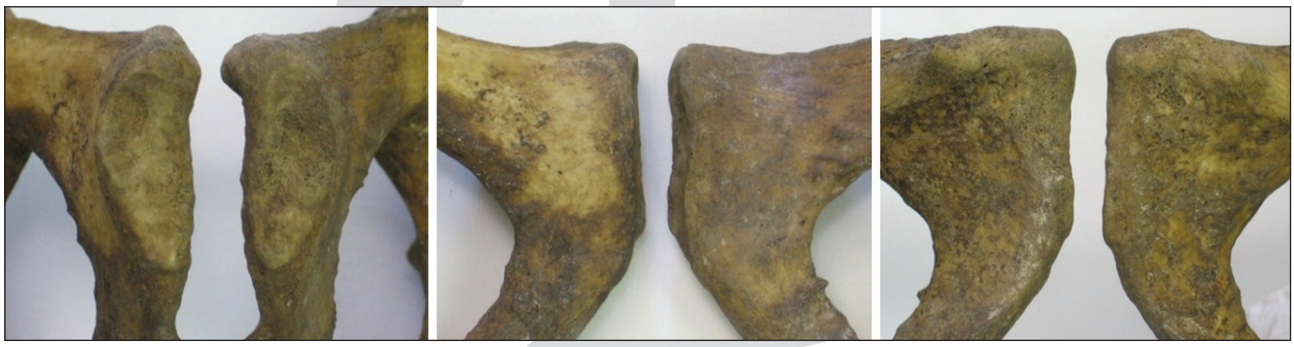
Surface of the symphysis face is mainly flat, with the remaining segments of horizontal ridges, and the first signs of depression in upper or lower segment, granulated, the ridges on pubic tubercle are completely missing. Dorsal rim is with highlighted plateau, ventral wall completely developed. Both extremities are entirely and clearly defined. Completely established frame around the entire symphysis. Tendon and ligamentous ossification processes on ventral rim, with development of valves on dorsal rim. Ventral rampart is mainly missing completely (Pictures 5a, 5b and 5c).

Phase 6 (age 25 – 50)

Symphysal face without ridge, flat, with completely formed frame which is elevated mildly above the surface on dorsal and ventral rim. Face surface generally shows minor or major depression – sinking. Irregular depression might occur in the upper part of ventral margin. Upper and lower extremities are better defined. Valves on dorsal margin are equal, with first signs of their forming on ventral side. Tendon and ligamentous ossification processes are more expressed. Ventral rampart is missing (Pictures 6a, 6b and 6c).

Phase 7 (age 37 – 60)

Surface of symphysis face shows the signs of porosity and development of new bone, which give it irregular and mildly toothed appearance, sometimes with irregular dimples in the lower part or generally. Face frame is irregular, sometimes toothed, with first signs of its erosion – disappear-



Picture 6a. Face *Picture 6b. Back side* *Picture 6c. Front side*

Picture 7a. Face *Picture 7b. Back side* *Picture 7c. Front side*

ing on dorsal margin, while ventral wall becomes wider, irregular and sometimes knotty in the upper part. Bone structure of upper and lower extremities is disappearing, ligament processes are more expressed, valves on ventral margin are more expressed, irregular (Pictures 7a, 7b and 7c).

Phase 8 (age 40 – 70)

Symphysal face shows further deepening, higher porosity and irregularity, frame of the symphysis face is disintegrated – disappearing, ventral and dorsal margin are also disintegrated and disappearing, broken on certain spots. Upper and lower extremity are merged and disintegrated more – disappearing, valves are irregular and toothed (Pictures 8a, 8b and 8c).

Phase 9 (age 50 – 80)

Face of the pubic symphysis looks deformed, worn off, toothed, with minor or major furrows and visible porosity and loss of bone mass as well as the signs of uncontrolled production of bone nods at new bone. Almost complete erosion of dorsal and ventral wall, with disappearing symphysis rim, and sustained irregular, toothed bone processes. Lower and upper extremities are entirely disintegrated – merged into the environment. Distortions at symphysis face are intensified with older age (Picture 9a, 9b and 9c).

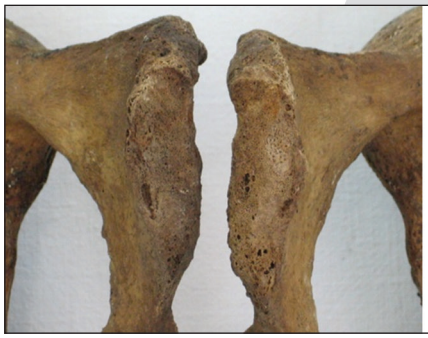
4. Conclusions

All twelve defined morphological features and related phases of their development morphological changes significantly correlate with the aging process. Morphological features with its phases at M7 (forming the upper extremity and M11 (porosity, face of the pubic symphysis worn off, emphasized and toothed face frame) correlate the best with the aging process.

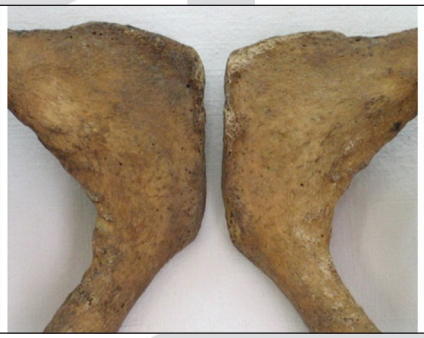
Analysed morphological changes of each pubic symphysis phase within certain morphological features significantly correlate with the aging process.

Morphological changes of pubic symphysis at BH male population are specific for that population.

The assessment led to development of brand new methods comprising 9 phases, with clearly expressed and methodologically explained morphological features, whose applicability would be tested among other populations of the world.

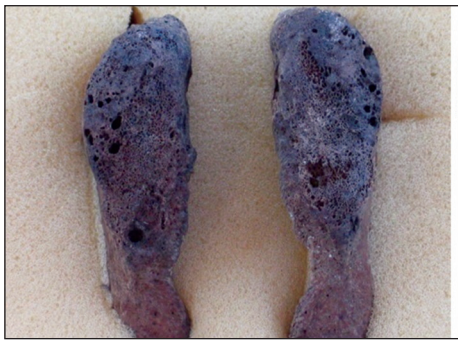


Picture 8a. Face

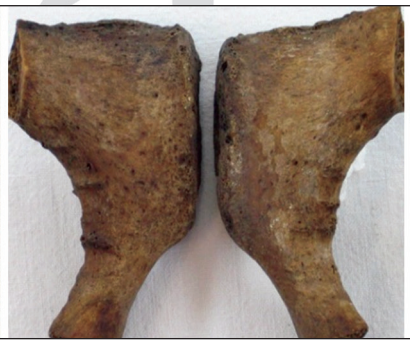


Picture 8b. Back side

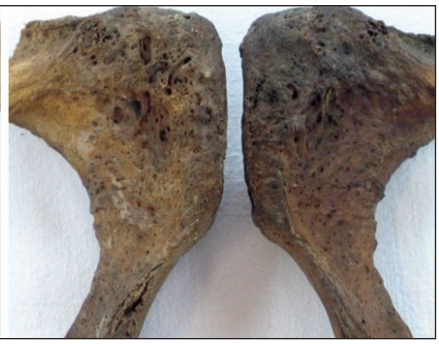
Picture 8c. Front side



Picture 9a. Face



Picture 9b. Back side



Picture 9c. Front side

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Is there a correlation between white blood cell count and insulin resistance in a Korean pediatric obesity?

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Abstract

Objectives: We aimed to investigate correlations between the White Blood Cell (WBC) count and insulin resistance (IR) in a Korean pediatric overweight and obese population.

Methods: 453 obese children (226 boys, 227 girls, aged 10-12 years) were included. Acquired measures included body mass index (BMI), body fat percentage (BF %), waist circumference, blood pressure, fasting glucose and insulin, hemoglobin, WBC count, total cholesterol (TC), triglycerides (TG), and high density lipoprotein-cholesterol (HDL-C). IR was calculated using the Homeostasis model assessment (HOMA).

Results: In boys, WBC count was positively correlated with BMI (r=0.21), waist circumference (r=0.19), BF % (r=0.28), total cholesterol (r=0.25), triglycerides (r=0.25), and diastolic blood pressure (r=0.19). In girls, WBC count was positively correlated with BMI (r=0.17), waist circumference (r=0.17), BF % (r=0.22), triglycerides (r=0.18), and systolic blood pressure (r=0.16). In multiple regression analysis with WBC count as the dependent variable, BF %, triglycerides, and total cholesterol emerged as determinants of WBC count (R^2 = 0.130 in boys, R^2 = 0.067 in girls) independently for age.

Conclusions. WBC count may not be correlated with IR in Korean pediatric obesity.

Key words: Insulin resistance, Pediatric obesity, White blood cell count

Introduction

Obesity is a risk factor for diabetes and cardiovascular disease, and its prevalence is increasing worldwide. Insulin resistance (IR) related with obesity is a main contributor to diabetes. IR of pediatric obesity can progress into adolescence, and highly correlates with type 2 diabetes or cardiovascular disease in adulthood. The prevalence rate of diabetes is globally increasing along with obesity, with the number of people known to have diabetes predicted to exceed 300 million by 2025.

IR is related to various inflammatory markers (1-3). Obesity is an inflammatory process. Increased proinflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP) and white blood cell (WBC) count represent dysfunctions of vascular endothelial cells, and are indicative of a chronic state of low grade inflammation. Inflammatory markers have been strongly correlated with IR and obesity in children as well as adults (4-6). WBC count in the peripheral blood can be used as an inflammatory marker and is a meaningful surrogate marker of childhood inflammation related with obesity and IR.

The WBC count is a fairly inexpensive and frequently performed procedure that can provide important information. However, little is known concerning the correlations between childhood IR and WBC count in the peripheral blood. Therefore, this study aimed to investigate correlations between the WBC count, which is the easily-measurable inflammation maker and homeostasis model assessment of IR (HOMA-IR), in a Korean pediatric overweight and obese population.

Materials and Methods

Subjects

Study of Seoul Obese School Children (SSOS)

This study was designed to identify the prevalence of risk factors and relationship between risk

factors and metabolic profiles in obese schoolchildren in Seoul. All primary schools in Seoul were invited to join the study. Among them, 15 elementary schools were selected by order of their responses. The target study subjects were overweight and obese students, over 85th percentile of age, gender specific Korean BMI references (7) in annual school examination. Anthropometric measurement, laboratory test, and a questionnaire were administered to the enrolled children (the child and his/her parents provided consent). A team consisting of five medical doctors, three nurses and three assistants were sent to the school for the measurements. After all the examinations, one doctor consulted students who were regarded as having serious medical or behavioral difficulties. This study (SSOS) was performed as a part of a children's obesity-related disease management program conducted by Seoul School Health Promotion Center, Study Group of Childhood Obesity, and Obesity Clinic in Kangbuk Samsung Medical Center and Inje University Sanggye Paik Hospital. This study was approved by Institutional Review Board of Inje University Sanggye Paik Hospital.

Total of 453 primary school children (226 boys, 227 girls, aged 10-12 years old) were enrolled. In this study, children with established diabetes, hypertension, recent infection, suspected endocrinological disease and who had not fasted for at least 9 hours were excluded.

Study Procedures

Anthropometric and blood pressure measurements, physical examinations

Height (cm) was measured to the last complete millimeter, with the subjects barefoot, using a wall-mounted stadiometer. Weight (kg) was measured to 100 g on a calibrated digital electric scale. BMI was calculated as kg/m^2. BMI percentile was calculated using Korean Children BMI Chart according to age and gender (7). Waist circumference was measured by a trained individual, to the nearest 0.1 cm, at the midpoint between the bottom of the rib cage and the top of the iliac crest with the subjects standing, their weight equally distributed on both feet, their arms at their sides, and head facing straight forward. Body fat percentage (BF %) was measured by bioelectrical impedance analysis (Inbody 720; Biospace, Seoul, Korea)(8).

Blood pressure (BP; mmHg) was measured in a seated position after relaxation for longer than 5 min using an automated oscillometric BP recorder (Dinamap procure 100; GE Medical System, Buckinghamshire, UK)(9). Arm circumference was measured and the appropriate cuff size was used. Seated BP was taken twice in succession with a 1-min interval from both arms, and additional measurement was performed in right arm if BP difference was more than 10 mmHg. The mean of the measurements were used in all analyses.

Biochemical measurements

After a fast of at least 9 hours, blood samples were drawn from an antecubital vein. Total cholesterol, glucose, high density lipoprotein-cholesterol (HDL-C), triglycerides, hemoglobin, hematocrit, and WBC count were measured using a model 747 automatic analyzer (Hitachi, Tokyo, Japan) and fasting serum insulin was determined by immunoradiometric assay (Biosource, Nivelles, Belgium). The HOMA-IR index was used where HOMA-IR = fasting glucose (mmol/L) x fasting insulin (μU/mL) / 22.5 (10).

Statistical analysis

The data are presented as mean ± standard deviation (SD) or the absolute number (percentages). Differences between boys and girls were assessed using t-tests. One-way ANOVA test was used to compare the obesity-related metabolic variables according to WBC count quartiles group. Correlation analyses were separately conducted after adjustment for age in boys and girls. Multiple linear regression analysis was conducted to reveal the explanatory variable. The level of significance for statistical tests was $p < 0.05$. All statistical analyses were performed using SAS Version 9.1.3, Korea.

Results

Demographic characteristics of subjects are shown in Table 1. The 453 subjects comprised 226 boys and 227 girls. In boys, mean age was 10.7 ± 0.9 years and mean BMI was 25.9 ± 2.5 kg/m^2. In girls, mean age was 10.3 ± 0.8 years and mean BMI was 24.6 ± 2.8 kg/m^2. Mean peripheral blood WBC count was 8186 ± 1814.6 cells/μL (range: 4820~20250 cells/μL). Age, height, weight, BMI, waist circum-

ference, hemoglobin and systolic BP showed significant differences by gender. BF %, hematocrit, WBC count, fasting glucose, insulin, HOMA-IR, total cholesterol, triglycerides, HDL-C, and diastolic BP did not show significant differences by gender (Table 1).

The differences in obesity-related metabolic variables after classification based on the quartile WBC count groups are shown in Tables 2 and 3. In boys, BF %, total cholesterol, triglycerides, and HDL-C were markedly elevated in the group with the higher WBC count (Table 2). In girls, BMI, waist circumference, and BF % were elevated in the group with the higher WBC count (Table 3).

Table 1. Basic characteristics of study subjects

Variables	Total (n=453) Mean ± SD	Boys(n=226) Mean ± SD	Girls(n=227) Mean ± SD	P*
Age (y)	10.5±0.9	10.7±0.9	10.3±0.8	<0.001
Height (cm)	149.0±8.1	151.0±8.5	147.1±7.4	<0.001
Weight (kg)	56.7±10.8	59.6±10.8	53.8±10.0	<0.001
BMI (kg/m^2)	25.2±2.7	25.9±2.5	24.6±2.8	<0.001
Waist circumference (cm)	84.8±7.3	86.3±6.9	83.3±7.4	<0.001
Body fat percent (%)	37.5±5.1	37.2±5.6	37.8±4.7	0.246
Hemoglobin (g/dL)	13.8±0.6	13.8±0.6	13.7±0.7	0.008
Hematocrit (%)	41.3±1.9	41.4±1.8	41.1±2.0	0.230
WBC count (cells/μL)	8186.9±1814.6	8292.5±1672.2	8081.8±1944.0	0.217
Fasting plasma glucose (mg/dL)	87.0±7.3	87.2±6.6	86.9±8.0	0.538
Insulin (μIU/mL)	18.9±13.1	18.1±13.5	19.7±13.1	0.196
HOMA-IR	4.1±3.1	3.9±3.1	4.3±3.2	0.262
Total Cholesterol (mg/dL)	182.7±31.1	183.4±31.0	182.1±31.2	0.651
Triglycerides (mg/dL)	131.1±64.5	131.7±68.7	130.5±60.1	0.842
High-density lipoprotein cholesterol (mg/dL)	55.7±12.4	56.6±12.3	54.8±12.3	0.127
Systolic BP (mm/Hg)	112.8±13.0	114.7±13.7	110.9±12.0	0.002
Diastolic BP (mm/Hg)	63.7±7.1	64.1±7.3	63.2±6.9	0.159

**P value was by t-test.*
Body fat percent missing value = 64
Body mass index (BMI) percentile (No/%): 85-90 percentile (23/5); 90-95 percentile (72/16); 95-100 percentile (358/79)
HOMA-IR; homeostasis model assessment-estimated insulin resistance
WBC; white blood cell, BP; blood pressure

Table 2. Anthropometric index and obesity related metabolic markers according to WBC quartiles in boys

Variables	WBC quartiles in boys(n=226)				
	≤7007(n=56)	7007<≤8115(n=57)	8115<≤9407(n=57)	>9407(n=56)	P
BMI (kg/m^2)	25.3±2.3	25.9±2.1	25.9±2.8	26.5±2.5	0.078
Waist circumference (cm)	84.6±7.0	86.8±6.2	85.8±7.9	88.0±6.4	0.069
Body fat percent (%)	34.5±5.4	37.6±4.4	37.7±5.9	39.1±5.5	0.001
Fasting plasma glucose (mg/dL)	88.2±6.6	87.3±6.4	87.5±6.7	85.8±6.8	0.289
Insulin* (μIU/mL)	2.72±0.44	273±0.52	2.80±0.45	2.79±0.51	0.753
HOMA-IR*	1.1±0.4	1.2±0.5	1.2±0.4	1.2±0.5	0.872
Total Cholesterol (mg/dL)	170.3±28.0	184.6±32.8	183.4±28.7	195.3±30.1	<0.001
Triglycerides (mg/dL)	109.2±55.7	125.1±63.9	137.6±78.0	154.8±68.9	0.004
High-density lipoprotein cholesterol (mg/dL)	59.5±14.7	54.7±10.3	58.9±12.6	53.3±10.4	0.014
Systolic BP (mm/Hg)	112.7±13.5	115.0±13.6	115.3±13.7	115.6±14.3	0.683
Diastolic BP (mm/Hg)	62.2±6.6	63.7±7.5	65.1±8.1	65.5±6.8	0.066

**Insulin, HOMA-IR were log transformed.*

Table 3. Anthropometric index and obesity related metabolic markers according to WBC quartiles in girls

Variables	WBC quartiles in girls(n=227)				
	≤6870(n=57)	6870<≤7740(n=58)	7740<≤9020(n=56)	>9020(n=56)	P
BMI (kg/m^2)	24.1±2.0	23.9±2.0	25.5±3.1	25.0±3.5	0.006
Waist circumference (cm)	82.6±6.7	81.3±5.7	84.9±8.3	84.5±8.2	0.030
Body fat percent (%)	36.9±3.6	36.7±4.6	38.3±4.6	39.5±5.3	0.008
Fasting plasma glucose (mg/dL)	87.9±12.9	86.5±5.7	86.1±5.7	86.7±5.2	0.674
Insulin* (μIU/mL)	2.81±0.50	2,78±0.70	2.84±0.60	2.83±0.54	0.953
HOMA-IR*	1.2±0.5	1.2±0.7	1.2±0.6	1.2±0.5	0.962
Total Cholesterol (mg/dL)	180.0±28.5	180.3±34.2	181.5±32.1	186.7±30.1	0.636
Triglycerides(mg/dL)	114.1±45.0	134.9±76.2	134.0±58.7	139.0±54.1	0.120
High-density lipoprotein cholesterol (mg/dL)	55.2±13.4	53.9±10.5	54.5±13.1	55.7±12.5	0.875
Systolic BP (mm/Hg)	109.5±11.4	109.8±13.3	110.5±11.4	113.9±11.4	0.181
Diastolic BP (mm/Hg)	62.2±7.5	62.6±6.7	62.6±5.7	65.4±7.2	0.055

**Insulin, HOMA-IR were log transformed.*

Table 4. Correlations between WBC count and obesity related markers after adjusting for age

Variables	Boys		Girls		Total	
	Correlation, r	P*	Correlation, r	P*	Correlation, r	P*
BMI (kg/m^2)	0.21	0.004	0.17	0.015	0.19	<0.001
Waist circumference (cm)	0.19	0.008	0.17	0.015	0.19	<0.001
Body fat percent (%)	0.28	<0.001	0.22	0.002	0.24	<0.001
Fasting plasma glucose(mg/dL)	-0.03	0.614	-0.05	0.465	-0.05	0.375
Insulin† (μIU/mL)	0.12	0.106	0.006	0.930	0.05	0.331
HOMA-IR†	0.11	0.149	0.000	0.995	0.05	0.422
Total Cholesterol(mg/dL)	0.25	<0.001	0.09	0.189	0.17	0.001
Triglycerides (mg/dL)	0.25	0.001	0.18	0.012	0.21	<0.001
High-density lipoprotein cholesterol (mg/dL)	-0.09	0.204	0.03	0.655	-0.02	0.667
Systolic BP (mm/Hg)	0.09	0.204	0.16	0.023	0.13	0.009
Diastolic BP (mm/Hg)	0.19	0.008	0.10	0.139	0.15	0.003

**P value < 0.05 by partial correlation analysis*
†Insulin, HOMA-IR were log transformed.

We used the partial correlation analysis after adjustment for age in boys and girls, respectively (Table 4). In boys, WBC count was positively correlated with BMI, waist circumference, BF %, total cholesterol, triglycerides, and diastolic BP. In girls, WBC count was positively correlated with BMI, waist circumference, BF %, total cholesterol, triglycerides, systolic BP, and diastolic BP. There was no significant correlation between WBC count and HOMA-IR as an IR index. BF % showed the highest correlation with WBC count in both genders. In multiple regression analysis with WBC count as the dependent variable, BF % ($\beta = 0.169$; $P = 0.030$ in boys, $\beta = 0.164$; $P = 0.039$ in girls), triglycerides ($\beta = 0.140$; $P = 0.056$ in boys, $\beta = 0.155$; $P = 0.026$ in girls), and total cholesterol ($\beta = 0.168$; $P = 0.022$ in boys) emerged as determinants of WBC count ($R^2 = 0.130$ in boys, $R^2 = 0.067$ in girls) independently for age (Tables 5 and 6).

Table 5. Multiple linear regression analysis in boys

Variables	B	Std. Error	ß	P
BMI (kg/m^2)	26.116	103.530	0.038	0.801
Waist circumference (cm)	12.345	35.569	0.050	0.729
Body fat percent (%)	51.638	23.649	0.169	0.030
Total Cholesterol (mg/dL)	9.102	3.933	0.168	0.022
Triglycerides (mg/dL)	3.444	1.787	0.140	0.056
Diastolic BP (mm/Hg)	20.410	16.647	0.088	0.222

Model for boys; $R^2 = 0.158$, Adjusted $R^2 = 0.130$, constant = 1136.683
Dependent variable is WBC count.
Independent variables are BMI, waist circumference, body fat percent, total cholesterol, triglycerides, and diastolic BP.

Table 6. Multiple linear regression analysis in girls

Variables	B	Std. Error	ß	P
BMI (kg/m^2)	8.845	83.380	0.013	0.916
Waist circumference (cm)	16.004	31.328	0.060	0.610
Body fat percent (%)	69.606	33.407	0.164	0.039
Triglycerides (mg/dL)	5.208	2.323	0.155	0.026
Systolic BP (mm/Hg)	14.781	11.825	0.091	0.213

Model for girls; $R^2 = 0.090$, Adjusted $R^2 = 0.067$, constant = 1557.686
Dependent variable is WBC count.
Independent variables are BMI, waist circumference, body fat percent, triglycerides, and diastolic BP.

Discussion

In this study, there was no significant correlation evidence between WBC count and IR in Korean pediatric obese subjects. However, WBC count showed a significant correlation with the obesity-related and metabolic variables, which are body fat percentage, total cholesterol, and triglycerides. Kim *et al*.(11) reported that 10-19 year old overweight girls showed no correlations between WBC count and IR. Another study by Lee *et al*.(12) showed that there was a significant correlation between high sensitivity (hs) CRP and IR in children, and explained that their results were assumed to be caused by relatively low sensitivity of WBC count for inflammation, compared to other inflammatory markers such as hs-CRP. Significant correlations between WBC count and IR have been well described in adults (13-16). In addition to WBC count, other various inflammatory markers such as hs-CRP, IL-6, and TNF-α have been confirmed to have correlations with insulin resistance in adults (17, 18).

When our study subjects were divided into four quartile groups based on WBC count, BF %, total cholesterol, triglycerides, and HDL-C tended to increase as the WBC quartiles increased in boys, and BMI, waist circumference, and BF % tended to increase in girls. This was similar to the study outcomes reported by Kelishadie *et al*.(19), in a study of 326 obese children in which increased BMI, waist circumference, triglycerides, total cholesterol, and low density lipoprotein-cholesterol were reported as WBC quartiles increased.

WBC count is highly correlated with risk factors of cardiovascular disease, and most of the studies on adults reported significant correlation between the factors of metabolic syndrome and WBC count. Both Heish *et al.*(20), who studied 1,657 young Japanese students 14-19 years of age, and Kim *et al* (21), who studied young Korean students, reported WBC counts were highly correlated with some factors of metabolic syndrome in adolescents. In this study, BMI, waist circumference, BF %, total cholesterol, triglycerides, and diastolic BP in boys, and BMI, waist circumference, BF %, triglycerides, and systolic BP in girls correlated with WBC count. Obesity is a low grade inflammatory state, and there is a strong relationship between inflammation and obesity (21).

In this study, the average WBC count was 8,186 cells/μL in the overweight children at 10.5 years of mean age. This level is higher than the average WBC count of previous studies in obese girls at 15.6 years of age, whose WBC count was 6,964 cells/μL (11,12) Our study subjects was relatively younger than the subjects in other studies and obese children, which the younger children have tended to have a higher WBC count. We thought this was the reason showed the higher mean WBC count at our study.

There are some limitations in our study. This study was cross-sectional design and so could not accurately assess a causal relationship. Prospective cohort studies are needed to establish the cause-and-effect relationship. Secondly, instead of the euglycemic-hyperinsulinemic clamp method, the HOMA-IR index based on fasting glucose and insulin was used. However, the use of euglycemic-hyperinsulinemic clamp method is inappropriate for large-scale clinical or epidemiological studies. Because the HOMA-IR index is highly correlated with IR, it was more appropriate for our designs. Thirdly, the subjects in this study could not be representative of all Korean children. Fourthly, it is hard to know whether the subjects were undergoing puberty since the Tanner stage was not used. Lastly, other factors that could affect the number of WBC count, such as smoking or alcohol drinking, were not considered, although the influence would likely be minimal since the subjects were in primary school.

Conclusion

WBC count might not be correlated with IR in obese children, but WBC count was positively correlated metabolic markers of obesity. Among the metabolic markers, obesity, BMI, waist circumference, BF %, total cholesterol, triglycerides, and diastolic BP showed significant correlations with WBC count in boys, and BMI, waist circumference, BF %, triglycerides, and systolic BP in girls were correlated with WBC count. Moreover, BF % and total cholesterol had the highest correlation in boys, and BF % and triglycerides had the highest correlation in girls. Further studies on causal relationship between WBC count and pediatric obesity are needed in the future.

Acknowledgement

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Evaluation of obstruction stage in acute exacerbation of chronic obstructive lung disease Prospective control study

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Abstract

Introduction: chronic obstructive lung disease is one of leading causes of illness and mortality in developed countires. Patients with frequent accute worsening have more expressed loss of lung function what leads to invalidity and sudden death. Aims of this research were: estimate the opstruction according to markers in time of accute exacerbation and 6 weeks after stabilisation of illness.

Respondents and methods: this prospective control research included 50 respondents treated from 1. 3. 2010. till 1. 3. 2011 for pulmonal diseases Tuzla. Estimation of opstruction is determined by testing of pulmonal function with spirometer (Jaeger masterlab). Gas analysis in artherial blood (PaO2 and PaCO2, SaO2) were measured by analysator (ABL, 620, Medical radiometer). Standard statistical package for social researches was used for data processing, (SPSS) version 10.0.

Results: 50 respondents with average age 67 ± 11 were analysed. The most of them were in age of > 70 (n=31). In total sample of HOPB respondents, 39 (78%) were men and 11 were women. At the moment of testing, 16 of respondents were smokers. 46 (92%) of respondents were negative in ventolin test. Significant differences in values were evident such as: values of functional breading parameters and gas analysis in repeated measurements: (t0, t1, t2). Values in stabile phase are statistically in significant difference regarding time of acute agzacerebation (t0:t1 v.s. FEV1: FEV2; ANOVA for repeated measurements; P=0.002).

During stabilization time and after AE, respiratory function significantly and irreversibly declined (negative trend >-4; FEV3). PCO2 values were significantly higher in AE while values of PO2 were significantly lower in statistical comparement with measurements in clinical stabilization phase. Differences in PO2 and PCO2 markers were not evident in stabile phase and after stabilization of AEHOPB. Low values of PO2 and PCO2, increased values of PCO2 represent strong predictor of HOPB outcome.

Conclusion: after AEHOPB statistically irreversible significant decline of respiratory function takes place which confirms small reversibility of respiratory function. The rate of decline FEV1 is good parameter of illness progression and mortality. AE marks significantly higher values of PCO2 as well as statistically significant lower values of PO2 in comparing with clinically stabile condition which represents insecure indicators of AEHOPB. Marker differences are not evident in PO2 and PCO2 markers are not evident in stabile phase and after stabilisation of AE. These parameters confirm chronical, irreversible HOPB process.

Key words: obstruction, spirometry, chronic obstructive lung disease

Introduction

Chronic obstructive lung disease (HOPB) is clinical entity characterized by progressive obstruction of airways which is not completely reversible and is caused by inappropriate inflammation response on longterm exposure to harmful particles and gases according to global initiative for HOPB (1). Prevalence of HOPB is 3-17% in developed and 13-27% in undeveloped countries. HOPB is multicomponent and multisystemic disease that starts with defect of morphology and function of lungs, and has progressive course and difficult acute deteriorations. Acute agzacerebations represent acute deteriora-

tions of stabile disease typical symptoms such as: intense and frequent cough, increased amount and purulence of mucus, progression of heavy breading and increased detoriation of general condition (2). Patients with frequent AEHOPB have increased loss of pulmonal function and life quality, frequent hospitalizations, what is related to increased risk of letal outcome (Aaron i sar., 2002). Testings have pointed out that most of patients with HOPB see their doctors in higher stage of disease i.e. disease is being diagnosed only after a bigger loss of pulmonal function (2). Apart from loss of pulmonary function, partial pressure PO2 also decreases, lesser than 9,3 kPa (hypoxemy), saturations of hemoglobin with oxygen, lesser than 0,94, with or without increased values od PCO2, over 6,3 kPa (hypercapnia) in arterial blood. Basic symptom of HOPB is difficulty in breading process (dyspnea). Aims of research: evaluate obstruction difficulty according to markers in time AE and 6 weeks later after illness stabilization.

Respondents and methods

50 treated patients were included in prospective, control research from 1.3.2010 till 1.3.2011 in pulmonary disease clinic UKC Tuzla, who were confirmed with clinical diagnosis of HOPB and instructed to hospital because of AE. Research was divided in 3 time intervals: t0 – spyrometrical parameters (FEV1, FEV1/FVC), markers of artherial blood gas analysis were determined previously in stabile phase od disease, t1 – spyrometrical parameters and gas analysis were determined on first and second day of hospitalization in all respondents who were experimental group in time of AE and t2 – spyrometrical parameters and markers of gas analysis were conducted in time of stabilization of HOPB six weeks after all signs of AE were taken care of. The most important parameters of HOPB with FEV1 were post bronchodilatational relation FEV1/FVC < 0.70. the assessment of opstruction level was determined by spirometer testing of pulmonal function (Jaeger Masterlab). Gas analysis in artherial blood (PaO2 i PaCO2, SaO2) were measured by analysator (Abl, 620, Medical Radiometer). BMI was calculated on basis of body mass in kilograms and body surface in square meters (weigh k/height m² = 21-25 kg/m2). Ergometric load test were conducted, "six minutes walking test" or 6MTH, which is used in diagnostics of dyspnea level. Statistical data processing: Standard statistical package for social researches was used for data processing, (SPSS) version 10.0. In statistical data processing standard methods of descriptive statistics were conducted. χ2-test and t-test were used for testing of statistical significance in difference between chosen variables. In order to test relation between level of dyspnea according to MRC scale and significant segments and scores SGRQ, nonparametric Spermans correlation was used (concerning that MRC scale is ordinary type). In order to test relation between dyspnea level and BMI, 6MTH significant segments and scores, Person's correlation was used. Univariate and multivariate analysis of variance with linear and logistic regressive analysis – ANOVA was used for multivariate correlation analysis.

Results

The largest number of treated for AEHOPB in pulmonary clinic are in age > 70 (n=31). Significant number of respondents are in age > 55 years (n=34).

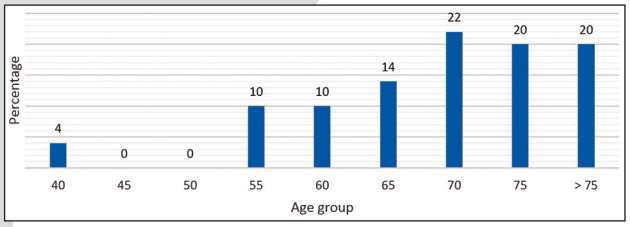

Figure 1. Distribution of all respondents (n=50) in total sample according to age subgroups

Men were frequently ill 39 (78%).

There are significant differences for values; 6 minutes walking test, functional parameters of breathing according to GOLD in repeated measurements (t0, t1, t2).

In AEHOPB values of PCO2 were significantly higher while values of PO2 were statistically much lower in comparing to measurements in phase of clinical stabilization. Fall of partial pressure of oxygen and increasment of partial pressure of CO2 could be insecure indicators of AEHOPB.

Values in stabile phase are significantly different regarding time of AE (t0:t1 v.s. FEV1: FEV2; ANOVA for repeated measurements; P=0.002).

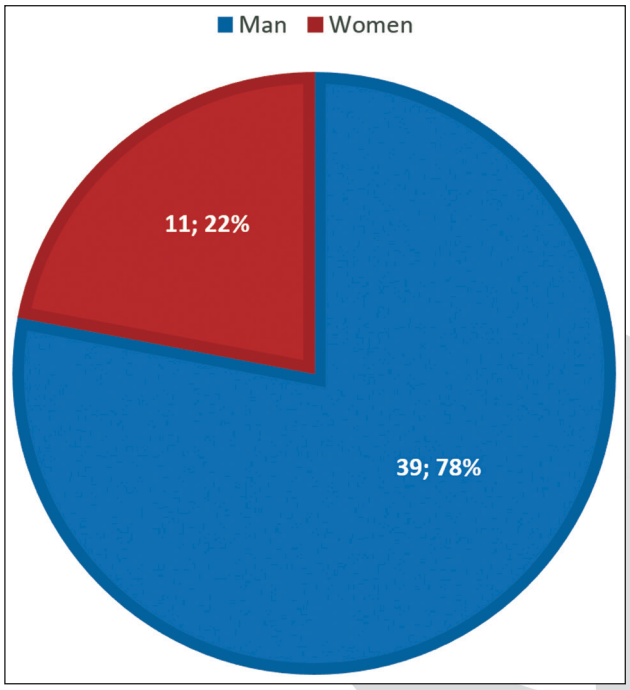

Figure 2. Structure of respondents according to sex

In time of stabilization after AE respiratory function irreversibly and significantly fell (negative trend >-4; FEV3).

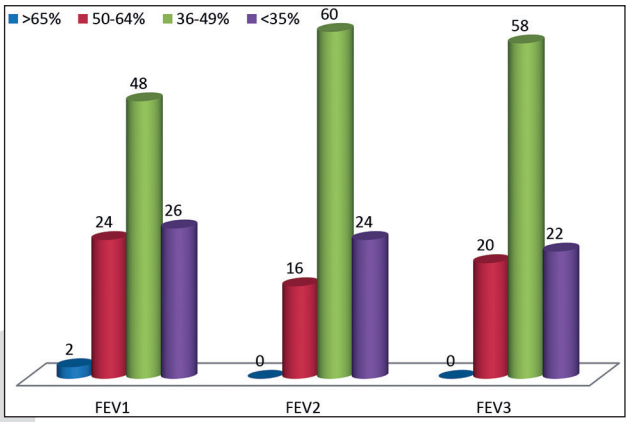

Figure 3. Comparative display of differences in values FEV1 in repeated measurements

Values of 6 minutes walking test in stabile phase were significantly different in statistics regarding time of AE (ANOVA for repeated measurements; P=0.014). in time of stabilization after AEHOPB impression was that functional capacity for activity was improving significantly what provides possibility of choice for respiratory physical rehabilitation, gradually (positive trend >+4 respondents; P= 0.009).

Table 1. Evaluation of complication level according to HOPB markers in previously stabile time, time of acute cerebration on day of hospitalization and time of stabilization

Markers	Measurement time	Maximum	Minimum	AS±SD*	p-values**
6-minutes walking test	t0	30.00	375.00	180.18±86.42	
	t1	25.00	300.00	125.38±60.32	0.014
	t2	25.00	350.00	158.38±90.62	
pCO2	t0	30.30	73.00	44.72±8.49	
	t1	17.00	74.50	47.00±10.35	0.879
	t2	32.80	60.00	44.56±6.85	
pO2	t0	32.10	89.00	59.95±10.74	
	t1	34.00	77.80	56.26±9.42	0.382
	t2	35.20	84.90	61.21±9.17	
	Measurement time	Maximum values	Minimum values	Median (int. range)	
GOLD[1]	t0	1	4	3 (3-4)	
	t1	2	4	3 (3-4)	0.002
	t2	2	4	3 (3-3)	

Table 2. Comparative values of opstruction markers: PCO2 and PO2 in time of egzacerebation and in time of stabilisation.

Marker	AS±SD	Average difference	Interval of trust 95%	p-value
PCO2 in egzacerebation	47.01±10.35			
PCO2 in stabilisation	44.56±6,85	2.45	0.18 do 4.72	0.035
PO2 in egzacerebation	56.26±9.42			
PO2 after stabilisation	61.21±9,17	-4.95	-7.47do 2 .44	0.001

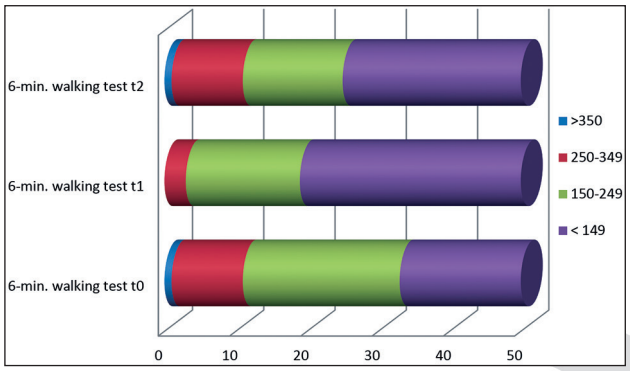

Figure 4. Comparative display of differences in values of 6 minutes walking test in (t0, t1, t2)

There were not statistical connection of BMI and FEV1 in any time of measurement. BMI index in our sample is not indicative mortality factor.

Discussion

HOPB nowadays represents global public health problem with high morbidity and mortality rate. Apart from social and economical significance, epidemiological studies on uts frequency are legging behind in researches on other health issues (3). During 2010 and 2011 there were 1770 with this disease on pulmonary clinic and 350 of them were with HOPB. Chronic obstructive pulmonary disease was leading cause of illness and hospitalizations. Results in English national study conducted in 2010 show that 842 100 out of 50 million people were with HOPB diagnose, approximately 1 person out of 59(4). Results in our research have pointed out that HOPB was frequently diagnosed in male population in Tuzla county between 40 and 86 age of life, and the largest number of tested respondents were in age of >70 (n =31), with large of extremely large stadium of HOPB according to Gold (III stadium n=37, IV stadium n=13). Several biomarkers that evaluate HOPB and effects of therapeutical treatment were legalized such as FEV1 global marker of HOPB. FEV1 is insufficient, not reflecting completely the level of HOPB, so legalization of new biomarkers (biological, physiological and symptomatic) was inevitable. In HOPB, especially in AE there is certain fall in pressure PO2, lower than 9,3 kPa (hypoxemia), saturation of hemoglobin with oxygen, lower than 0,94, with or without increased values of PCO2, over 6,3 kPa (hyperkapnia) in arterial blood, which is significant in evaluation of level and flow of disease. Low values of PO2, increased PCO2 represent strong predictor of HOPB outcome. Results of our research have pointed out that there was a significant fall of PO2 (P=0,001) and increase in PCO2 in in repeated measurements in AE, what would represent insecure marker AE-HOPB. These data indicate that the largest number were with large or extremely large HOPB i.e. in distant phase of chronical respiratory insufficiency. Lethal outcome risk in patients with HOPB is often evaluated by usage of one physiological variable, forced expiratory volume in first second (FEV1)(5). In time of stabilisation after AE respiratory function irreversibly and significantly declines (negative trend >-4; FEV1-t2). The fall of FEV1 was evident in 8 cases, 1 case was with increasment of value while in 41 cases the change was not evident in class according to Gold. Therefore, dominated cases werewith decreasment of FEV1 values and this change was statistically significant (Wilcoxon Z=-2.33; p=0.02). The rate of fall of FEV1 is good indicator of progression of disease and mortality. This research didn't show significant difference in values of BMI where average values were (od 27,08 ± 5,17 do 26,13 ± 5,9. P=0,087) in repeated measurements and does not

Table 3. Display of correlation of BMI and FEV1 in repeated measurements (t0-t1-t2)

BMI	correlation	FEV1 t0	FEV1 t1	FEV1 t2
BMI t0	Coefficient correlation p-value	0.084 0.560	-0.008 0.956	-0.087 0.549
BMI t1	Coefficient correlation p-value	-0.068 0.641	-0.0134 0.352	-0.211 0.142
BMI t2	Coefficient correlation p-value	-0.080 0.582	-0.128 0.376	-0.206 0.151

represent mortality predictor. Low index of body mass is related to increased mortality risk (6-7). Patients that could not handle ergometrical tests of load due to level of illness, 6MTH test was conducted and used in diagnostics of level of dyspnea. Shorter walk without dyspnea is with worse illness prognosis. Values of 6 minutes walking test in our research in 'stabile phase were significantly different in statistics comparing with time of acute egzacerebation (ANOVA for repeated measurements; P=0.014).

Conclusions

HOPB was frequently diagnosed in male population in Tuzla county between 40 and 86 age of life. Differences in markers PO2 and PCO2 in stabile phase and after stabilization of AE are not evident. These parameters confirm chronic, irreversible process of HOPB (stability of markers). In stabilization time after AEHOPB statistically irreversible significant fall of respiratory function is evident. There are statistically significant higher values in 6 minutes walking test (158.88 m±(90,62 m) with average difference of 33,5 meters regarding time of AE in stabile phase of HOPB. BMI is not significant negative mortality predictive factor.

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Relation between Premature Atherosclerosis and Lipoprotein (a) in Non-Alcoholic Fatty Liver Disease

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Abstract

Background: The relationship between non-alcoholic fatty liver disease (NAFLD) and premature atherosclerosis has been previously demonstrated in many studies. Although the literature comprises various data about the effect of lipoprotein (a) (Lp[a]) on premature atherosclerosis, the role of Lp(a) in premature atherosclerosis in those with NAFLD is unclear.

Aim: To investigate the relationship between premature atherosclerosis and Lp (a) in patients with NAFLD

Study design: A prospective study

Methods: The present study included 144 subjects (males under the age of 55 years and females under the age of 65 years) who underwent ultrasonography to determine hepatosteatosis of any reason, had no diagnosis of malignancy, had no history of heavy alcohol consumption, and had no primary liver disease. Reviewing retrospectively, the subjects were divided into three groups according to the degree of hepatosteatosis as Grade 0 (no hepatosteatosis), Grade 1 (mild hepatosteatosis), and Grade 2-3 (moderate-severe hepatosteatosis). Age, gender, demographic characteristics, and laboratory parameters of the subjects were recorded. Blood pressure, body weight and height, and waist circumference were measured. Metabolic syndrome was diagnosed according to the International Diabetes Federation (IDF) criteria. All subjects underwent carotid Doppler ultrasonography of the neck for carotid intima-media thickness (cIMT) measurement. Lp(a) level was measured using nephelometric method.

Results: A positive correlation was observed between the grade of hepatosteatosis and the right and left cIMTs. Significantly higher cIMT was observed with increasing grade of hepatosteatosis ($p<0.05$). Lp(a) level was significantly higher in the Grade 2-3 hepatosteatosis group than in the Grade 0 hepatosteatosis group ($p=0.046$). Taking 0.8 mm as the cut-off value for cIMT in those with NAFLD, no significant difference was determined between those with cIMT of >0.8 mm and of <0.8 mm in terms of Lp(a) level ($p=0.516$). Age over 50 years (odds ratio [OR]=27.19, 95% confidence interval [CI], 2.66–285.00, $p=0.005$), presence of grade 2-3 hepatosteatosis (OR=15.56, 95% CI, 3.20–76.60, $p=0.001$) and age between 40-50 years (OR=11.13, 95% CI, 1.07-115.25, $p=0.043$) were found as independent risk factors likely to influence premature in NAFLD.

Conclusion: In the light of current data, we concluded that NAFLD was associated with premature atherosclerosis and that severe hepatosteatosis together with an age over 40 years had an impact on premature atherosclerosis independent of other cardiovascular risk factors. We concluded that Lp(a) had no impact on increased premature atherosclerosis in the presence of hepatosteatosis.

Key word: Non-Alcoholic Fatty Liver Disease, Premature Atherosclerosis, Lipoprotein(a)

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a clinical spectrum with an increasing importance in recent times, in which histological features of alcoholic hepatosteatosis are observed in individuals not consuming alcohol. NAFLD may present with numerous pathological conditions of the liver including simple steatosis, non-alcoholic steatohepatitis, advanced fibrosis, and cirrhosis (1). Metabolic syndrome (MetS) is a fatal endocrinopathy that begins with insulin resistance and is associated with atherosclerotic disorders and in which abdominal obesity, glucose intolerance, and systemic disorders such as diabetes mellitus (DM), dyslipidemia, hypertension (HT), and

coronary artery disease coexist (2). It is thought that NAFLD occurs as the hepatic reflection of MetS secondary to the inflammatory substances released by visceral adipose tissue, which is enlarged in MetS and functions as an endocrine organ (3). Hence, due to this close relationship, it could be considered that the parameters of MetS have an impact on increased atherosclerosis in the presence of NAFLD. However, recent studies have suggested that NAFLD is an independent risk factor for coronary artery diseases (4). There are large-scale meta-analyses demonstrating that, in addition to manifest coronary artery disease, subclinical atherosclerosis and atherosclerotic carotid plaques are also strongly associated with NAFLD (5). Some of the earlier studies have emphasized that NAFLD displays its independent effect on subclinical atherosclerosis by managing the inflammatory process through hepatokines and adipokines (fetuin-A, fibroblast growth factor 21, and selenoprotein P) released by the liver (6,7).

Lipoprotein(a) (Lp[a]) is the modified large glycoprotein form of low-density lipoprotein (LDL), where apolipoprotein(a) (apo[a]) binds to apolipoprotein B via disulfide bonds (8). Many functions of the Lp(a) is mediated by apo(a), which is the plasmin-like component. Owing to its structural similarity to plasminogen, apo(a) inhibits fibrinolysis by competing with the molecules and cells to which plasminogen is bound. Plasmin formation and fibrinolysis are inhibited with the impairment in the activation of plasminogen (9). Moreover, it stimulates the formation of foamy cells by binding to the high-sensitive receptors on the macrophages and leads to smooth muscle activation in the arterial wall (10,11). Lp(a) has similar plasma concentration in males and females; high plasma Lp(a) levels are regulated by genetic variations in the apo(a) gene. For this reason, although Lp(a) shows racial differences, 30%-60% of the variations in Lp(a) levels in a population is attributed to the polymorphism of this gene region (12,13). Lp(a) is also sensitive to oxidative modifications; this is considered to cause progression of atherosclerotic lesions and permanently increased carotid intima-media thickness (cIMT) by leading to formation of proinflammatory and proatherogenic phospholipids, oxysterols, and oxidized lipid-protein compounds in the Lp(a) (14).

Measurement of cIMT is a non-invasive method that is widely used to evaluate atherosclerosis. The relationship of increased cIMT with atherosclerosis and cardiovascular diseases is well known. Increased cIMT has also been demonstrated in NAFLD patients with disease progression from simple steatosis to steatohepatitis (15,16).

The present study aimed to demonstrate the relationship between premature atherosclerosis and Lp(a) in patients with NAFLD.

Patients and Methods

This prospective study included patients (male patients under the age of 55 years and female patients under the age of 65 years) who were admitted to Trakya University Medical Faculty Hospital and diagnosed with NAFLD and healthy individuals without NAFLD as a control group. Patients who had a history of heavy alcohol consumption (>30g/day for males and >20 g/day for females), patients diagnosed with a malignancy, patients presented for acute illnesses, patients having inflammation, tissue necrosis, trauma, rheumatoid arthritis, vasculitis syndrome, Crohn's disease, ulcerative colitis, deep anemia, severe hypothyroidism, nephrotic syndrome, atrial fibrillation, or moderate-to-severe valvular disease, patients who received statins (within the last 5 days), patients with low functional capacity, patients with a liver disease, and patients with a history of documented atherosclerosis or biliary disease, and pregnant females were excluded. Patients were included in the study on the condition that they underwent whole abdominal ultrasonography (US) and/or hepatobiliary US according to the hospital medical records. The present study was approved by the Ethics Committee of Trakya University Faculty of Medicine (date: 17/07/2013, decision no: 16/10, and approval protocol number: 2013/128). The study subjects were informed about the study and their written consents were obtained.

All patients were classified according to the presence and degree of ultrasonographic hepatosteatosis. Accordingly, they were divided into 3 subgroups as Grade 0 (those with no hepatosteatosis), Grade 1 (those with mild hepatosteatosis), and Grade 2-3 (those with moderate-severe hepatosteatosis) groups. Age, gender and general de-

mographic characteristics of all subjects were recorded. Demographic characteristics and detailed anamnesis were obtained and all subjects underwent detailed cardiovascular examination after 10 minutes of resting period. Body weight, height, waist circumference, and blood pressure of all subjects were measured. Body mass index (BMI) was calculated by Quetlet's index (kg/m^2). The patients were diagnosed with MetS based on the International Diabetes Federation (IDF) criteria (17).

Analysis of Lp(a) was performed with the nephelometric method using the IMMAGE® Immunochemistry Systems (Beckman Coulter, Inc., CA, USA) and Siemens N Latex Lp(a) Reagent commercial kit (Siemens Healthcare Diagnostics GmbH, Marburg, Germany). The reference range was 0-30 mg/dL.

Radiological Examination

All study subjects were manually examined through the mid area of both main carotid arteries using high-resolution B-mode US (Esaote Vısıon MyLab 60, Italy) with a linear 7.5 MHz probe while the patients were in the supine position with the neck positioned to the opposite side at an angle of 20°. The carotid artery was scanned longitudinally to obtain the optimal image. The highest cIMT was measured only at the posterior wall (the distant wall) in the mid-area of both carotid arteries as the basis. All measurements were performed by the same radiologist.

Statistical Analysis

Data analysis was performed using the IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics of the data were expressed as mean, standard deviation, ratio, and frequency. Normality of data was analyzed using the Kolmogorov-Smirnov test. For normally distributed quantitative variables, analysis of variance (ANOVA) test was used for comparison of three groups and independent sample t-test was used for two group comparisons. For non-normally distributed variables, comparison of three groups was performed using Kruskal-Wallis test and two group comparisons were performed by Mann-Whitney U test. Chi-square test was used for the analysis of qualitative data. Correlation analysis was performed using Spearman's correlation analysis. Multivariable logistic regression analysis was performed to demonstrate the independent effects of MetS parameters and presence of NAFLD on the cIMT.

Results

The present study included 144 subjects in whom whole abdominal US and/or hepatobiliary US was performed. Of the study patients, 46.5% were female, 53.5% were male, 40.3% had HT, 18.8% had DM, 34.7% had dyslipidemia, 19.4% had positive family history of cardiovascular disease 34.7% had history of smoking, and 41.0% had MetS. Grade 0 hepatosteatosis was determined in 44.4% (n=64, control group), Grade 1 hepatosteatosis was determined in 41.7% (n=60), and Grade 2-3 hepatosteatosis was determined in 13.9% (n=20) of the study subjects (Table 1).

Table 1. Demographic and clinical characteristics of the study subjects

Characteristics	
Age, year	46.48±9.13
Gender	
Female	67 (46.5)
Male	77 (53.5)
HT	58 (40.3)
Dyslipidemia	50 (34.7)
Family history of cardiovascular disease	28 (19.4)
Smoking	50 (34.7)
BMI, kg/m^2	28.5±5.23
MetS	59 (41.0)
DM	27 (18.8)
Hepatosteatosis	
Grade 0	64 (44.4)
Grade 1	60 (41.7)
Grade 2-3	20 (13.9)

HT, hypertension; NAFLD, non-alcoholic fatty liver disease; BMI, body mass index; MetS, metabolic syndrome; DM, diabetes mellitus.

Data are presented as mean±standard deviation or number (percentage), where appropriate.

According to hepatosteatosis grades, the rate of HT was significantly lower in Grade 0 hepatosteatosis group than in Grade 1 and Grade 2-3 hepatosteatosis groups ($p<0.001$). The rate of DM was significantly lower in Grade 0 hepatosteatosis group than in Grade 2-3 hepatosteatosis group

(p<0.001), whereas it was significantly higher in Grade 1 hepatosteatosis group than in Grade 2-3 hepatosteatosis group (p<0.001). The rate of family history of NAFLD was significantly lower in Grade 0 and Grade 1 hepatosteatosis groups than in Grade 2-3 hepatosteatosis group (p<0.001). While the rate of dyslipidemia was significantly lower in Grade 0 hepatosteatosis group than in Grade 1 and Grade 2-3 hepatosteatosis groups (p<0.001), it was significantly higher in Grade 2-3 hepatosteatosis group than in Grade 1 hepatosteatosis group (p<0.001). MetS was significantly less prevalent in Grade 0 hepatosteatosis group than in Grade 1 and Grade 2-3 hepatosteatosis groups (p<0.001), whereas it was significantly more prevalent in Grade 2-3 hepatosteatosis group than in Grade 1 hepatosteatosis group (p<0.001). No significant difference was determined among the three groups in terms of frequency of smoking (p=0.460; Table 2).

No significant difference was determined among the Grade 0, Grade 1, and Grade 2-3 hepatosteatosis groups in terms of age (p=0.064) and gender (p=0.829). The BMI and left and right cIMTs were significantly higher in Grade 2-3 hepatosteatosis group than in Grade 0 and Grade 1 hepatosteatosis groups (p<0.001) and they were significantly higher in Grade 1 hepatosteatosis group than in Grade 0 hepatosteatosis group (p<0.001). No significant difference was determined among the three groups in terms of high-sensitivity C-reactive protein (hs-CRP) values (p=0.721). Lp(a) value was significantly higher in Grade 2-3 hepatosteatosis group than in Grade 0 hepatosteatosis group (p=0.046) (Table 3).

Correlation analysis performed to investigate the relationship of NAFLD with the right and left

Table 2. Clinical characteristics of Grade 0, Grade 1, Grade 2-3 hepatosteatosis groups

	Hepatosteatosis			
Characteristics	**Grade 0 (n=64) n (%)**	**Grade 1 (n=60) n (%)**	**Grade 2-3 (n=20) n (%)**	**p**
HT	13 (20.3)*‡	29 (48.3)	16 (80.0)	<0.001
DM	5 (10.4)*	24 (36.9)*	14 (35.0)	<0.001
Family History	6 (9.4)*	12 (20.0)*	10 (50.0)	<0.001
Dyslipidemia	9 (14.1)*‡	25 (41.7)*	16 (80.0)	<0.001
MetS	9 (14.1)*‡	33 (55.0)*	17 (85.0)	<0.001
Smoking	23 (35.9)	18 (30.0)	9 (45.0)	0.460

HT, hypertension; DM, diabetes mellitus; MetS, metabolic syndrome.
*Chi-square test: *Different from Grade 2-3 group at p<0.05; ‡ different from Grade 1 group at p<0.05.*

Table 3. Clinical, biochemical, and ultrasonographic findings of Grade 0, Grade 1, Grade 2-3 hepatosteatosis groups

	Hepatosteatosis			
	Grade 0 (n=64)	**Grade 1 (n=60)**	**Grade 2-3 (n=20)**	**p**
Age, year, Mean±SD	44.5±9.4‡	47.6±9.0	49.1±9.0	0.064
Gender, n (%)				
Female	28 (43.8)	29 (48.3)	10 (50.0)	0.829
Male	36 (56.3)	31 (51.7)	10 (50.0)	
BMI, kg/m^2, Mean±SD	25.6±4.4*‡	30.1±4.6*	32.7±4.4	<0.001
Right cIMT, mm, Mean±SD	0.616±0.139*‡	0.698±0.119*	0.839±0.161	<0.001
Left cIMT, mm, Mean±SD	0.622±0.157*‡	0.698±0.115*	0.871±0.207	<0.001
hs-CRP, mg/dL, Mean±SD	0.67±1.16	0.75±1.14	0.39±0.12	0.721
Lp(a), mg/dL, Mean±SD	13.6±7.06*	16.1±13.1	28.3±30.0	0.046

SD, standard deviation; BMI, body mass index; cIMT, carotid intima media thickness; hs-CRP, high sensitivity C-reactive protein; Lp(a), lipoprotein(a).
ANOVA/Kruskal-Wallis (Mann-Whitney U test).
**different from Grade 2-3 group at p<0.05; ‡different from Grade 1 group at p<0.05.*

cIMTs and Lp(a) revealed that NAFLD was significantly, moderately and positively correlated with the right cIMT (r=0.467, p<0.001) and with the left cIMT (r=0.463, p<0.001); and NAFLD was significantly and positively but weakly correlated with Lp(a) (r=0.184, p=0.027).

Taking 0.8 mm as the cut-off value, the NAFLD group with cIMT of <0.8 mm and the NAFLD group with cIMT of ≥0.8 mm was compared in terms of Lp(a) levels; no significant difference was determined between the groups in terms of the mean Lp(a) levels (18.3±18.6 mm and 20.7±20.5; p=0.516; Table 4).

Owing to the impacts of MetS parameters, presence of NAFLD, age, presence of DM, elevated LDL, and elevated BMI on atherosclerosis, a multivariable logistic regression analysis was performed taking a cIMT of 0.8 mm as the cut-off value to demonstrate their independent effects on premature atherosclerosis. The analysis revealed that age between 40-50 years, age ≥50 years, and presence of Grade 2-3 hepatosteatosis were found as independent risk factors likely to influence premature atherosclerosis (odds ratio [OR=11.13], 95% confidence interval [CI], 1.07–115.25, p=0.043; OR=27.19, 95%CI, 2.66-285.00, p=0.005; OR=15.56, 95%CI,

Table 4. Multivariable logistic regression analysis of the parameters having an impact on increased carotid intima media thickness

	OR (cIMT≥0.8 mm/cIMT<0.8 mm)	**95% CI**	**p**
Hepatosteatosis			
Grade0	1	-	
Grade1	2.30	0.73-7.19	0.151
Grade2-3	15.56	3.20-76.60	**0.001**
Age, years			
<40	1	-	
40-50	11.13	1.07-115.25	**0.043**
≥50	27.19	2.66-285.00	**0.005**
HT			
Absent	1	-	
Present	0.85	0.31-2.35	0.764
Hypertriglyceridemia			
Absent	1	-	
Present	0.60	0.21-1.68	0.331
Low HDL			
Absent	1	-	
Present	0.82	0.32-2.10	0.688
DM			
Absent	1	-	
Present	0.65	0.20-2.06	0.467
Waist circumference			
Normal	1	-	
High	1.13	0.31-4.06	0.841
LDL			
<130	1	-	
>130	1.02	0.40-2.54	0.966
BMI			
<30	1	-	
>30	1.69	0.62-4.55	0.299

OR, odds ratio; cIMT, carotid intima media thickness; CI, confidence interval; HT, hypertension; HDL, high-density lipoprotein; DM, diabetes mellitus; LDL, low-density lipoprotein; BMI, body mass index.

3.20-76.60, p=0.001; respectively). HT, DM, low high-density lipoprotein (HDL), hypertriglyceridemia, high waist circumference, and elevated LDL and BMI values were not found to be significant independent risk factors for the increase in cIMT (Table 4).

Discussion

The role of Lp(a), which is a particle known for 50 years, in the development of atherosclerosis has remained unclear due to the technical difficulties in measurements and to the negative studies in the literature (18). Various studies have demonstrated that Lp(a) is effective in the development of clinical or subclinical atherosclerosis (19-22). On the contrary, some studies in the literature have reported that increased atherosclerosis may also be observed in case of low Lp(a) levels and that Lp(a) may cause atherosclerotic foamy cell formation by acting as a scavenger particularly at its low concentrations (23). NAFLD is a clinical condition involving 30% of the population and having already documented close relationship with atherosclerosis today. Hence, a meta-analysis of 34 studies conducted between 1965 and 2015 reported NAFLD as an independent risk factor for coronary artery disease, hypertension, and atherosclerosis (24). However, the literature has gaps regarding what kind of role such a mysterious molecule as Lp(a) plays in atherosclerosis development in NAFLD. For this reason, the present study aimed to assess whether Lp(a) had any relationship with the atherosclerosis in NAFLD.

In the present study, the rates of female and male patients were 46.5% and 56.5%, respectively, and there was no significant difference between the study groups (Grade 0, 1, and 2-3 hepatosteatosis groups) in terms of gender. Although there is male dominance in some studies to eliminate the hormonal effects on NAFLD, the present study established a balanced study population in terms of gender for reflecting the general population (25). Moreover, since there might be radiologist-related bias between Grades 2 and Grade 3 hepatosteatosis, advanced hepatosteatosis was gathered in a single group as Grade 2-3. In the present study, the mean age of whole study population was 46.48±9.13 years; there was no significant difference between the three study groups in terms of age. In an earlier study conducted by Puig et al. (26) investigating the effects of age and NAFLD on atherosclerosis in morbid obese subjects, the mean age of the NAFLD group was 45.04±9.34 years and the mean BMI was 44.5±3.0 kg/m^2. They demonstrated age and presence of NAFLD to be independent risk factors in predicting carotid atherosclerosis (26). Although the mean age of our subjects was similar to those reported in the above-mentioned study, the mean BMI of the whole study population (28.52±5.23 kg/m^2) and the mean BMI of the advanced hepatosteatosis (Grade 2-3) group (32.7±4.4 kg/m^2) were significantly lower than that reported in the above-mentioned study. Dichotomizing the NAFLD subjects according to the cut-off value of 0.8 mm for cIMT, we found no significant difference between the Lp(a) values of the groups with cIMT of <0.8 mm and of >0.8 mm. Accordingly, we thought that Lp(a) had no significant effect on increased premature atherosclerosis observed in NAFLD. In the literature, some large-scale, powerful, and well-designed studies on the effect of Lp(a) on atherosclerosis have stated that Lp(a) has no effect on cardiovascular diseases or stroke and have suggested that genetic and technical reasons, as well as acute phase reactions against Lp(a) are quite likely reasons of these unfavorable outcomes (18). Contrarily, the present study analyzed pure Lp(a) level from serum; however, detailed genetic analysis according to different apo(a) isoforms of Lp(a) was not performed. Thus, some of the apo(a) isoforms found in different sizes in the Lp(a) may be effective in atherogenesis in NAFLD patients even in cases of low Lp(a) concentrations. On the contrary, Calmarza et al. (27) conducted a study in 172 asymptomatic Spanish male population and found no relationship between Lp(a) and cIMT, a finding which is in accordance with the findings of the present study, even with detailed analysis performed according to the different apo(a) isoforms and phenotypes. Tarantino et al. (28) found no significant relationship between Lp(a) and increased cIMT in obese NAFLD patients, which is a similar finding to the results of the present study, and stated that Lp(a) had no direct effect on increased cIMT (28). It should be noted that the above-mentioned studies were conducted on

different gender and ethnicity groups. In the present study, however, the study groups did not differ regarding the rates of males and females and particularly the subjects living in the Eastern Thrace were enrolled. The difference in the distribution of Lp(a) among certain ethnic groups is known (29). Thus, randomized, large-scale studies that would be conducted in different patient groups in the same region and supported by genetic background are required. The failure of earlier studies in determining a relationship between Lp(a) and atherosclerosis has been attributed to the studies being performed in small patient populations and the specimens being waited so long as frozen (30).

The present study was a prospective study; thus, the specimens were kept frozen for less than a year and were analyzed as soon as the enrolment of study population was completed. Moreover, different life styles (such as Mediterranean diet, exercise) of the study subjects were not taken into account and how Lp(a) would affect the premature atherosclerosis in NAFLD patients in the presence of these situations particularly together with predefined risk factors remains debatable. Additionally, considering significantly high Lp(a) value despite large standard deviation in Grade 2-3 hepatosteatosis group (Lp(a): 28.3±30.0 mg/dL), trying to explain premature atherosclerosis through the point of Lp(a) in a larger population of grade 2-3 hepatosteatosis patients with more homogenous distribution of Lp(a) level may provide more significant outcomes. The limited number of grade 2-3 hepatosteatosis patients in the present study might have led to the failure in determining a relationship with Lp(a).

In the light of available data, we concluded that NAFLD was associated with premature atherosclerosis and that particularly severe hepatosteatosis together with an age over 40 years had an effect on premature atherosclerosis independent of other cardiovascular risk factors. We also concluded that Lp(a) had no effect on increased premature atherosclerosis in the presence of hepatosteatosis.

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Comparative analysis of age assesment based on pubic symphysis

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Abstract

Introduction: One of the safest and most frequently used indicators of human skeletal age is morphological changes on face of the pubic symphysis. Morphological changes on pubic symphysis provide the most exact information on person's age at death. In the nineteenth century Abey (1858) noticed age variations in upper and lower part of the pubic symphysis face, as well as horizontal ridges on pubic symphysis face that change with aging, and that dorsal margin is formed before the ventral one. The first standard in assessment of age at death based on morphological features of pubic symphysis was set by the American anatomist Tood (1920, 1921). He defined the age related changes on pubic symphysis through 10 phases with related range of years. Suchey-Katz 1986 and Suchey-Brooks 1990 defined the method of pubic symphysis comprising six phases. Acsadi and Nemeskeri (1970) presented the age related changes on public symphysis through five phase.

The aim of this study was to compare the results of the age assessment of male skeletal remains according to BH nine-phase method and Suchey-Brooks model of 6 phases.

Material and methods: The sample comprised 101 pairs of male pubic symphysis, known year of birth and year of death. The age was determined by relatives, after seeing the identification document (birth certificate). The youngest person was aged 15 and the oldest 78.

Results: Methodologically, descriptive statistic was used, regression-correlation analysis and multiple regression model. Persons aged between 40 and 60 dominate in the sample. The average age for the whole sample is 43.72 years with standard deviation of 15.93 years. Half of persons from the sample were aged 48 or less, while the other half was over 48 years of age in the moment of death. The most frequent age of persons in the sample was 53 in the moment of death. Actual age and morphological features of pubic symphysis (M1-M12) with all phases of nine-phase model significantly correlate ($p<0.05$). At the same time, phases for all morphological features significantly correlate mutually. The average absolute deviation from the analysed sample is lower at the nine-phase model (5.193) compared to the age assessment utilizing Suchey-Brooks method of six phases (5.339). In addition, variation coefficient of age assessment of nine phases is lower (V-11.877) compared to the assessment utilizing Suchey-Brooks model (V-13.653). Method of age assessment of nine phases has a higher correlation level (0.971) with actual age than Suchey-Brooks model (0.914).

According to the results of the Wilcoxon test, comparison of actual age and estimated age according to the nine-phase model does not significantly deviate from the actual age at death (-0.879), while deviation of age assessment according to Suchey-Brooks model compared to actual age is much bigger (-1.628).

Conclusion: New method of nine phases for age assessment of skeletal remains enables more precise age assessment than Suchey-Brooks method.

Key words: Pubic symphysis, age assessment, nine-phase model, Suchey-Brooks model.

1. Introduction

Currently, forensic anthropology takes a leading role in the processes related to violation of human rights, war crime and terrorism investigation procedures. One of the safest and by forensics most frequently used indicators of age assessment of skeletal remains are age-related morphological changes on pubic symphysis.

Morphological changes on pubic symphysis face are surely one of the most reliable criteria which consistently follow the aging process. In the nine-

teenth century Abey (1858) noticed age variations in upper and lower part of pubic symphysis face, as well as horizontal ridges on pubic symphysis face which change with aging, and that dorsal margin of face is formed before the ventral one (1).

The first standards in the assessment of age at death based on morphological features of pubic symphysis were set by the American anatomist Tood (1920, 1921). He defined morphological changes on pubic symphysis at death through 10 phases, each phase having a defined range of years of age (2, 3).

Acsadi and Nemeskeri in 1970 presented the morphological changes on pubic symphysis in five phases, with certain range of years (4). Modifying the Todd's method, Suchey and Brooks (1986, 1990) developed pubic symphysis method for male persons on a large sample N=739. (5, 6). They presented morphological changes on pubic symphyses face through six phases, shown at the photos and plaster moulds. Each phase has a certain range of years.

In a court-anthropology processing of numerous human skeletal remains (over 15000) from the previous BH war, the assessment of their age at death utilizing Tood and Suchey-Brooks method did not give sufficiently precise results, in fact there were some cases of underestimated but also overestimated age at death (Simmons and associates, 1999; Tuco, 2007; Tuco and Sarajlić, 2010; Tuco, 2011). (7, 8, 9, 10). At the same time, observations and conclusions of other authors who were addressing the issue of age assessment of skeletal remains based on morphological features of pubic symphysis are that there are significant individual differences in skeleton morphology at one population, as well as among the populations, and that it is necessary to develop pubic symphysis model based on morphological features of pubic symphysis for certain population group (Ubelaker, 1989; Sinha and Gupta, 1995; Iscan, 1988; Baccino and associates, 1999; Bednarek, 2002; Schmitt, 2004; Đurić and associates, 2007; Xiping C and associates, 2008; Hens and associates, 2008). (11, 12, 13, 14, 15, 16, 17, 18, 19). The objectives of this study were to assess the age utilizing nine-phase method developed for BiH population by Tuco 2011 (11), assess the age based on Suchey-Brooks method (5) and correlate the results of assessed lifetime age between these two methods.

2. Material and methods

The research was conducted at the sample of 101 pairs of pubic symphysis of exhumed male skeletal remains of persons who disappeared during the previous was, aged between 15 and 78. The identity of persons was confirmed by DNA analysis.

Pubic symphysis that were complete and intact skeletized naturally, with proper orientation of left and right groin bone.

After that, morphological features of each pubic symphysis pair from the tested sample were compared with the defined morphological features of pubic symphysis of BiH male population nine-phase model and morphological features of Suchey-Brooks method.

The obtained results were analysed utilizing descriptive statistics, regression-correlation analysis and multiple regression model.

3. Results and discussion

The average age of the tested sample is 43.72 years, standard deviation 15,93 years. Half of the cases from the sample were aged 48 years or less, while the other half was over 36 years of age at death. Dominant cases in the sample were the persons aged at death from 40 to 60 years, while there were the least of persons aged 70 and more. Persons aged 53 at death were the most frequent in the sample (Table 1).

Table 1. Descriptive statistics for variable actual age at death

Variable: Actual age	
Average	43,72
Median	48
Mode	53
Standard deviation	15,93
Variation coefficient	36,44
Variation range	63
Minimum	15
Maximum	78
Standard average estimation error	1,585
Confidence interval for the average (95% CI)	40,578 – 46,587
Number of observations (*N*)	101
KS test	$z = 1{,}036$ $p = 0{,}049 < 0{,}05$

KS test – Kolmogorov-Smirnov test

Average absolute deviation of assessed age of the analysed sample utilizing nine-phase pubic symphysis model for bosnian-herzegovinian population has lower value (5.193), the same as variation coefficient (11.877) than at Suchey-Brooks method (average absolute deviation 5.339 and variation coefficient 13.653), compared to the actual lifetime age (Table 2).

Table 2. Absolute deviation per unit and variation coefficient of estimated age of the assessed sample for BH nine-phase model and Suchey-Brooks method

Assessment model	BiH nine phase model	S-B
Average absolute deviation	5,193	5,339
V	11,877	13,653

Variation coefficient – V, BiH – bosnian-herzegovinian Suchey-Brooks - S-B

Level of correlation between actual age at death and estimated age of the assessed sample is higher at BiH nine-phase model (0.971) than at Suchey-Brooks method (0.914), (Table 3).

4. Conclusions

All analysed morphological features of BiH nine-phase model of pubic symphysis with related phases significantly correlate with the aging process. Assessment of age at death utilizing pubic symphysis BiH nine-phase model has lower absolute deviation and lower variation coefficient of assessment compared to the actual lifetime age, than Suchey-Brooks method, which practically means that age assessment utilizing this model is more precise than original Suchey-Brooks method.

BiH nine-phase model of age assessment has higher correlation level with actual age compared to Suchey-Brooks method. Years range within the phases for BiH nine-phase model is smaller than at Suchey-Brooks method ranging from 3 to 30 years, while at Suchey-Brooks method it ranges between 8 to 52 years. Morphological changes of pubic symphysis at male bosnian-herzegovinian population are specific for that population.

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Table 3. Correlation of actual age at death and assessment of obtained values utilizing BiH nine-phase model and Suchey-Brooks method

		Actual age	S-B average given phase standard	BiH average nine phase model
Actual age	Pearson correlation coefficient	1	0,914	0,971
	P value		0,000	0,000
	N	101	101	101
S-B average given phase standard	Pearson correlation coefficient	0,914	1	0,935
	P value	0,000		0,000
	N	101	101	101
BiH average nine phase model	Pearson correlation coefficient	0,971	0,935	1
	P value	0,000	0,000	
	N	101	101	101

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Effect of Human Placental Extract on Quality of Life in Postmenopausal Woman

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Abstract

Objective: We aimed to evaluate the effect of Human placental extracts (HPE) on the quality of life (QOL) in postmenopausal Korean women aged over 40 years, using Women's Health Questionnaire (WHQ) and Nottingham Health Profile (NHP).

Methods: 100 volunteers over the age of 40 who had menopausal symptoms were recruited, and 60 women contributed to the analyses. The women were randomly assigned to receive subcutaneous injection of either placebo (normal saline) or HPE for 2 weeks by patient-blind method. To assess the QOL, we used WHQ and NHP translated into Korean. We applied WHQ and NHP at baseline, 2 weeks and 6 months after injections.

Results: 46 women participated in the final analysis (24 in the HPE group and 22 in the placebo group). The scores of WHQ and NHP were not significantly different between the HPE and the placebo groups at baseline. After 2 weeks, the score for sleep (NHP Part I) of HPE group was significantly improved (p=0.029). After follow-up period of 6 months, the score changes for pain (NHP Part I, p=0.048) and sleep (WHQ, p=0.004) of HPE group were significantly improved, but not in the placebo group.

Conclusion: HPE injections were partially effective in QOL improvement of postmenopausal women.

Key words: Human placental extracts, Quality of life, Postmenopausal women.

Introduction

Placenta obtained on delivery has been used traditionally for treatment of disease, such as anxiety disorder, epilepsy, dementia, chronic bronchitis, and general weakness in Korea. We could find some records about treatment with human placenta from Korean traditional herbal-medical textbook. The method to extract human placenta was developed in Japan, and Ministry of Health approved its clinical use in 1956. After approval, Human placental extracts (HPE) had been widely used in various clinical settings in Japan. HPE were known to have a lot of bio-active and therapeutic elements. So far, various growth factors, cytokines, hormones, peptides, lipids, nucleic acids, vitamins and minerals were identified (1, 2). In addition, HPE might contain unknown traces, and have the promising effect such as anti-inflammatory, anti-mutagenic, anti-anaphylactic and anti-oxidative (3, 4).

HPE began to be imported to Korea for treatment of chronic liver disease since 1994. Additionally, Korean physicians began using HPE to improve menopausal symptoms since 2003. The indications of HPE are now expanding to cover various disease entities, such as liver dysfunction, sexual dysfunction, ageing, fatigue syndrome and cosmetic problems of skin, although there are few evidence on HPE efficacy. Most of the studies were performed in animal or experimental based settings (5). Some authors reported the availability of HPE on the skin-whitening effect by anti-melanin action (6), and others insisted that HPE were effective on the hypopigmentation disorders by stimulation on melanocytes (7-9).

The objectives of the present study were to evaluate the effect of HPE on the quality of life in postmenopausal women aged over 40 years, using Women's Health Questionnaire (WHQ) and Nottingham Health Profile (NHP).

Material and Methods

Study participants

100 volunteers over the age of 40 who had menopausal symptoms were recruited in one university hospital in Seoul, Korea from July to August, 2007. We only included women who be-

came to be menopausal spontaneously within 5 years and who have more than 20 points in Kupperman's index. This study was approved by the Hospital Ethics Committee of Inje University Sanggye-Paik Hospital, Seoul, Korea.

After exclusion of women with a past history or current condition such as allergy to drug, cancer, thyroid disease, uncontrolled hypertension, complicated diabetes, uncontrolled dyslipidemia, neuropsychiatric disorder, systemic infections, hysterectomy, oophorectomy, hepatic or renal dysfunction, drug abuse, hormone therapy within 1 month, endometrial thickness over 5mm, abnormal Pap smear result within 6 months, vaginal bleeding and experience to be treated with HPE, 60 women contributed to the analyses. We randomly assigned subjects into 2 groups, which were HPE and placebo group. In addition, 14 participants were excluded for final analyses as one showed hematuria during the study, another one withdrew participation, and 12 did not complete questionnaire. Finally, we could assign 24 into HPE group and 22 into placebo group.

Study protocols

The women were randomly assigned to receive subcutaneous injection of either placebo (normal saline) or HPE for 2 weeks. From August to September, 2007, HPE or placebo was injected twice or three times weekly (6 times totally) by patient-blind method. To assess quality of life, we used WHQ and NHP, which were translated into Korean. We applied the 2 questionnaires for three times, which were at baseline, 2 weeks later and 6 months after injections. We measured pulse rate, body temperature, the height and weight of the women at baseline and calculated body mass index (BMI) (weight [kg]/ height [m^2]). Blood pressure was measured with a mercury blood pressure gauge in sitting position at rest. Laboratory variables assessed at baseline of the study using blood glucose, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum total bilirubin, lipid profile, serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH). In addition, we measured socioeconomic indicators such as marital status, monthly incomes and educational status. Marital status was divided into maintaining and non-maintaining group (unmarried, divorced, bereaved and separated).

Study tools

WHQ is a measure of mid-aged women's emotional and physical health (10). Since its publication in 1992 the WHQ has been widely used in multinational clinical trials, in epidemiological studies as well as in the evaluation of non-medical treatments. In particular the WHQ included quality of life measure in trials of hormonal preparations for peri- and postmenopausal women. The questionnaire was developed in English and standardized on a sample of women aged 45–65 years. It is reliable, valid and sensitive to detect change, and now available in 27 languages. The WHQ is a 36-item questionnaire assessing nine domains of physical and emotional health rated on four point scales. The nine domains are depressed mood (6 items), somatic symptoms (7 items), anxiety/fears (4 items), vasomotor symptoms (2 items), sleep problems (3 items), sexual behavior (3 items), menstrual symptoms (4 items), memory/ concentration (3 items) and attractiveness (3 items). The four point scales (yes definitely, yes sometimes, no not much, no not at all) are reduced to binary options (0/1) and the subscale items are summated and divided by the number of items in each subscale. We used 32 items except menstrual symptoms, translated into Korean, which was proved to have validity and reliability by Lee et al (11).

NHP is a two-part self-completed questionnaire. Part I assesses perceived health along six dimensions (Energy, Sleep, Emotional Reactions, Pain, Physical Mobility and Social Isolation). Each dimension consists of a number of statements which respondents are asked whether they apply to them or not. Each statement is weighted to produce a scoring system ranging from zero (no problems) to 100 (all the stated problems). Part II asks about the effect any ill-health has on seven areas of daily life. We used the NHP translated into Korean, which was proved to have validity and reliability by Moon et al (12).

Statistical analyses

We used SAS software for Windows version 9.1 (SAS institute, Cary, USA). We used χ^2-test and Fisher's exact test for comparison of HPE with placebo at baseline. We measured both pre and post injection scores, and difference of score changes to assess the effect of HPE on quality of life. We used Wilcoxon signed ranks test for comparison pre and post injection scores within groups, and Mann-

Whitney U test for the difference in score changes between baseline and after the study. All values are median (range) and a p value of less than 0.05 was considered to be statistically significant.

Results

Baseline clinical characteristics

In Table 1, 14 women did not complete the study because of several reasons as described above. Therefore, 46 women participated in the final analyses (24 in the HPE group and 22 in the placebo group). The age, weight, height, BMI and scores for WHQ and NHP were not significantly different between the HPE and placebo groups. In socioeconomic indicators, marital status and monthly incomes were not different, but educational levels were lower in placebo groups (p=0.018).

There were no serious adverse effects except only mild local symptoms such as pain on injection site, redness, itching, bruising. Additionally, one woman in the HPE group showed hematuria during the study period, and excluded from analysis. The incidence rate of adverse effects was not significantly different between two groups.

Short-term changes of quality of life after injections

To assess short-term changes of quality of life, we applied WHQ and NHP after 2 weeks from injections. The NHP Part I scores were significantly different in total Part I scores (before, 188.2 vs. after 2 weeks, 139.1, p=0.002), physical mobility (12.5 vs. 12.5, p=0.021), pain (25.0 vs. 12.5, p=0.005), sleep (40.0 vs. 20.0, p=0.029) and energy (66.7 vs. 33.3, p=0.018) in HPE group. In placebo group, total Part I scores (181.9 vs. 148.3, p=0.044) and

Table 1. Basic characteristics of study subjects (n=46).

Characteristic	HPE group (n=24)	Placebo group (n=22)	P value
Age(years, mean±SD)	54.6±2.0	53.9±2.3	0.442*
Weight(Kg)	55.7±5.9	57.2±5.3	0.295*
Height(Cm)	156.8±5.3	156.0±3.4	0.446*
BMI(Kg/m^2)	22.6±2.0	23.5±2.2	0.156*
Marital status, N(%)			0.659†
Married	17(70.8)	10(45.5)	
Unmarried	3(12.5)	3(13.6)	
non-response	4(16.7)	9(40.9)	
Degree of education, N(%)			0.018†
<middle school	2(8.3)	7(31.8)	
high school	14(58.3)	4(18.2)	
University	3(12.5)	2(9.1)	
non-response	5(20.8)	9(40.9)	
Income, N(%)			0.342†
<200(10 thousand won/month)	8(33.3)	7(31.8)	
200-400	7(29.2)	4(18.2)	
>400	4(16.7)	0	
non-response	5(20.8)	11(50.0)	
Women's Health Questionnaire(mean±SD)	83.6±15.5	77.5±14.6	0.222‡
Nottingham health profile(Ⅰ)(mean±SD)	214.2(12.5-478.9)	169.9(0-350.9)	0.276‡
Nottingham health profile(Ⅱ)(mean±SD)	2.6±2.3	2.2±2.4	0.488‡

HPE: Human placental extract, BMI: body mass index.
**by χ^2-test, †by Fisher's exact test, ‡by Wilcoxon signed ranks test.*

energy (50.0 vs. 33.3, p=0.017) were significantly different. There were no significant differences in the NHP Part II scores in both groups. On the other hand, the WHQ scores were not significantly different in each item in both groups (Table 2).

Long-term changes of quality of life after injections

To assess long-term changes of quality of life, we applied WHQ and NHP again after 6 months from injections. The NHP Part I scores were significantly different in total Part I scores (before, 188.2 vs. after 6 months, 128.9, p=0.002), physical mobility (12.5 vs. 12.5, p=0.038), pain (25.0 vs. 12.5, p=0.006), sleep (40.0 vs. 20.0, p=0.029), energy (66.7 vs. 33.3, p=0.018) and social isolation (20.0 vs. 0.0, p=0.033) in HPE group. On the other hand, in placebo group, there were no significant differences in each item. In addition, there were no significant differences in the NHP Part II scores in both groups. The WHQ scores were not significantly different in almost each item in both groups (Table 3).

Comparison for short-term changes of WHQ and NHP scores after injection

We compared score changes between HPE and placebo groups to assess the differences in two groups after 2 weeks from injections. The greater the score changes, the greater the improvement of quality of life. There were no significant score changes between HPE and placebo groups in NHP, except sleep (HPE, 0.0 vs. placebo, 0.0, p=0.033). In WHQ, items of cognitive difficulties (p=0.056) and sleep problem (p=0.077) seemed to be different marginally, but we could not find any more differences between two groups (Table 4).

Comparison for long-term changes of WHQ and NHP scores after injections

We compared score changes between HPE and placebo groups to assess the differences in two

Table 2. The comparison of treatment response between the two study groups after 2 weeks.

Measurements (No. of items)	HPE group, median(range)		P value*	Placebo group, median (range)		P value*
	Baseline	After 2 weeks		Baseline	After 2 weeks	
Nottingham health Profile(I)	188.2 (12.5-478.9)	139.1 (0-426.4)	0.002	181.9 (0-350.9)	148.3 (0-281.1)	0.044
physical mobility(8)	12.5(0-50)	12.5(0-50)	0.021	0.0(0-50)	0.0(0-37.5)	0.431
pain(8)	25.0(0-100)	12.5(0-87.5)	0.005	12.5(0.87.5)	12.5(0-62.5)	0.156
sleep(5)	40.0(0-80)	20.0(0-60)	0.029	20.0(0-60)	20.0(0-60)	0.366
energy(3)	66.7(0-100)	33.3(0-100)	0.018	50.0(0-100)	33.3(0-100)	0.017
social isolation(5)	20.0(0-100)	20.0(0-60)	0.069	20.0(0-100)	0.0(0-60)	0.499
emotional reaction(9)	33.3(0-88.9)	22.2(0-88.9)	0.252	44.4(0-88.9)	22.2(0-77.8)	0.051
Nottingham health Profile(II)(7)	2.0(0-7)	1.0(0-7)	0.090	1.0(0-7)	1.0(0-7)	0.101
Women's Health Questionnaire	82.0(51-107)	79.5(48-109)	0.136	80.5(50-98)	77.0(43-101)	0.548
somatic symptom(7)	19.0(7-26)	19.0(10-27)	0.221	16.0(7-24)	16.0(7-26)	0.215
depressed mood(7)	17.0(10-26)	16.5(9-23)	0.062	16.5(7-22)	15.5(7-20)	0.509
cognitive difficulties(3)	8.5(5-12)	8.5(4-12)	0.216	8.0(3-11)	9.0(3-12)	0.216
anxiety/fears(5)	12.0(8-17)	12.0(7-18)	0.776	11.0(5-17)	11.0(7-15)	0.599
sexual functioning(3)	7.0(0-12)	7.5(0-12)	0.924	7.0(1-12)	7.5(2-10)	0.676
vasomotor symptom(2)	6.0(2-8)	6.0(2-8)	0.371	6.5(2-8)	5.5(4-8)	0.082
sleep problem(3)	9.0(5-12)	8.0(5-12)	0.094	7.5(4-11)	8.5(3-12)	0.354
attraction(2)	5.0(2-9)	5.0(2-8)	0.668	5.0(2-8)	5.0(3-8)	0.147

**by Wilcoxon signed ranks test.*

Table 3. The comparison of treatment response between the two study groups after 6months.

Measurements (No. of items)	HPE group, median (range)		P value*	Placebo group, median (range)		P value*
	Baseline	After 6 months		Baseline	After 6 months	
Nottingham health Profile(I)	188.2 (12.5-478.9)	128.9 (0-330.9)	0.002	181.9 (0-350.9)	132.7 (23.6-321.1)	0.232
physical mobility(8)	12.5(0-50)	12.5(0-50)	0.038	0.0(0-50)	12.5(0-50)	0.785
pain(8)	25.0(0-100)	12.5(0-87.5)	0.006	12.5(0.87.5)	12.5(0-100)	0.824
sleep(5)	40.0(0-80)	20.0(0-80)	0.029	20.0(0-60)	20.0(0-80)	0.952
energy(3)	66.7(0-100)	33.3(0-100)	0.018	50.0(0-100)	33.3(0-100)	0.240
social isolation(5)	20.0(0-100)	0.0(0-80)	0.033	20.0(0-100)	0.0(0-60)	0.051
emotional reaction(9)	33.3(0-88.9)	22.2(0-66.7)	0.117	44.4(0-88.9)	22.2(0-55.6)	0.131
Nottingham health Profile(II)(7)	2.0(0-7)	1.0(0-6)	0.100	1.0(0-7)	2.0(0-7)	0.977
Women's Health Questionnaire	82.0(51-107)	80.0(50-105)	0.339	80.5(50-98)	83.0(45-100)	0.702
somatic symptom(7)	19.0(7-26)	19.0(9-27)	0.542	16.0(7-24)	18.0(8-24)	0.948
depressed mood(7)	17.0(10-26)	16.0(10-21)	0.081	16.5(7-22)	16.0(8-21)	0.746
cognitive difficulties(3)	8.5(5-12)	9.0(5-12)	0.486	8.0(3-11)	8.0(3-12)	1.000
anxiety/fears(5)	12.0(8-17)	12.0(8-17)	0.749	11.0(5-17)	12.0(7-16)	0.917
sexual functioning(3)	7.0(0-12)	7.5(0-11)	0.507	7.0(1-12)	9.0(3-11)	0.253
vasomotor symptom(2)	6.0(2-8)	6.0(3-8)	0.774	6.5(2-8)	6.0(4-8)	0.839
sleep problem(3)	9.0(5-12)	8.0(5-12)	0.090	7.5(4-11)	9.0(4-12)	0.024
attraction(2)	5.0(2-9)	5.0(2-7)	1.000	5.0(2-8)	5.0(3-8)	0.230

**by Wilcoxon signed ranks test.*

groups after 6 months from injections. There were no significant score changes between HPE and placebo groups, except pain (HPE, 12.5 vs. placebo, 0.0, p=0.048) of NHP Part I and sleep (HPE, 1.0 vs. placebo, -1.0, p=0.004) of WHQ (Table 5).

Discussion

Although there is few evidence about its effects so far, Human placental extracts (HPE) are now expanding to cover various disease entities, such as liver dysfunction, sexual dysfunction, aging, fatigue syndrome and cosmetic problems of skin. Earlier studies suggested that HPE has anti-inflammatory properties and HPE has been assumed to have anti-ageing action via fibroblast proliferation and growth-promoting effect (13, 14). In addition, some authors reported effects of HPE on chronic non-healing wounds (15, 16). Kong et al. suggested that menopausal symptoms and fatigue in middle-aged Korean women improved after 8 weeks of HPE treatment (17). But research evidence of these effects is still lacking. The quality of life, including not only objective, but also subjective feeling of well-being, is very broad concept. Therefore, evaluation of subjective well-being sense is important when we assess the quality of life. In this study, we investigated whether significant changes of the quality of life occur after HPE treatment, and we focused well-being sense mainly rather than bioactivity or analysis for sub-elements of HPE. As a short-term result, we could observe significant improvements of NHP Part I scores in total Part I scores (p=0.002), physical mobility (p=0.021), pain (p=0.005), sleep (=0.029) and energy (p=0.018) in HPE group. In placebo group, total Part I scores (p=0.044) and energy (p=0.017) were significantly different. These results seemed to be maintained after 6 months later. But with comparison for changes of WHQ and NHP scores between HPE and place-

Table 4. The comparison of short-term score change between the two study groups after 2 weeks.

Measurements (No. of items)	HPE group short-term score change, Median (range)	Placebo group short-term score change, Median (range)	P value*
Nottingham health Profile(I)	48.6(-86.6~249.1)	22.8(-69.4~187.9)	0.456
physical mobility(8)	0.0(-12.5~25.0)	0.0(-25.0~37.5)	0.324
pain(8)	12.5(-12.5~37.5)	0.0(-25.0~50.0)	0.427
sleep(5)	0.0(-20.0~80.0)	0.0(-40.0~20.0)	0.033
energy(3)	0.0(-33.3~44.5)	33.3(-33.3~100.0)	0.647
social isolation(5)	0.0(-40.0~60.0)	0.0(-40.0~60.0)	0.470
emotional reaction(9)	11.1(-33.4~44.5)	11.1(-33.4~55.6)	0.631
Nottingham health Profile(II)(7)	1.0(-6.0~6.0)	0.0(-1.0~4.0)	0.540
Women's Health Questionnaire	3.0(-28.0~21.0)	3.0(-20.0~17.0)	0.552
somatic symptom(7)	1.5(-12.0~6.0)	1.0(-4.0~7.0)	0.947
depressed mood(7)	2.0(-8.0~8.0)	0.0(-7.0~8.0)	0.347
cognitive difficulties(3)	0.0(-3.0~3.0)	-0.5(-5.0~3.0)	0.056
anxiety/fears(5)	0.0(-6.0~5.0)	0.0(-6.0~3.0)	0.947
sexual functioning(3)	0.0(-4.0~4.0)	0.5(-6.0~7.0)	0.797
vasomotor symptom(2)	0.0(-2.0~3.0)	0.5(-2.0~2.0)	0.378
sleep problem(3)	0.5(-3.0~6.0)	0.0(-5.0~3.0)	0.077
attraction(2)	0.0(-4.0~3.0)	-0.5(-3.0~4.0)	0.322

**by Mann-Whitney U test.*

bo groups after injections, we could only find partial score changes in sleep (p=0.033) of NHP part I as a short-term result, pain (p=0.048) and sleep (0.004) of NHQ as a long-term results. These might be due to high prevalence of pain and sleep disorder, and the other items such as physical mobility, emotional reaction, depressed mood, energy and anxiety were thought to be dispersed widely according to personal susceptibility. We have to consider other medical and social conditions such as comorbidities of osteoarthritis and other degenerative disease, therapeutic modality, degree of labor, personal susceptibility and medication history when evaluating pain. Similarly, the considerations for stress, emotional status, sleep quality and medication history are essential when we evaluate the HPE effect on sleep. Therefore, we could not conclude that the above changes were totally due to HPE injections.

The difference of score changes between WHQ and NHP were higher than expected, and these trends were sustained during the study period. It seems to be due to simplicity of NHP and complexity of WHQ.

We acknowledge some strengths and limitations of this work. To our knowledge this study is the first prospective one that assessed the effects of HPE on the quality of life in postmenopausal women. Furthermore, we rechecked the questionnaires after 6 months to find out the long-term effect of HPE.

The current study has several limitations. First is the lack of objective assessment on quality of life. We used only questionnaire scores to assess the quality of life change. Postmenopausal symptoms are subjective feeling, so we cannot conclude that the questionnaire score changes represent degree of quality of life exactly. Also it could be due to the placebo effect related to psychological support. Second, the results could not represent other socio-medical conditions that could impact on the quality of life of participants (11). Finally, the

Table 5. The comparison of long-term score change between the two study groups after 6 months.

Measurements (No. of items)	HPE group long-term score change, Median (range)	Placebo group long-term score change, Median (range)	P value*
Nottingham health Profile(I)	48.0(-115.5~293.6)	43.4(-206.2~245.0)	0.361
physical mobility(8)	0.0(-12.5~25.0)	0.0(-25.0~37.5)	0.135
pain(8)	12.5(-25.0~50.0)	0.0(-62.5~62.5)	0.048
sleep(5)	0.0(-20.0~40.0)	0.0(-40.0~60.0)	0.122
energy(3)	33.3(-33.3~66.7)	16.7(-66.7~100.0)	0.957
social isolation(5)	20.0(-40.0~80.0)	0.0(-40.0~60.0)	0.536
emotional reaction(9)	11.1(-33.3~77.8)	11.1(-44.5~66.7)	1.000
Nottingham health Profile(II)(7)	1.0(-5.0~5.0)	0.0(-5.0~5.0)	0.096
Women's Health Questionnaire	4.5(-30.0~34.0)	1.0(-47.0~18.0)	0.314
somatic symptom(7)	0.5(-14.0~10.0)	1.0(-10.0~7.0)	0.608
depressed mood(7)	2.0(-6.0~9.0)	-1.0(-11.0~9.0)	0.423
cognitive difficulties(3)	0.0(-5.0~3.0)	0.0(-6.0~3.0)	0.710
anxiety/fears(5)	0.0(-6.0~7.0)	0.0(-9.0~6.0)	0.813
sexual functioning(3)	0.0(-5.0~5.0)	-1.0(-7.0~8.0)	0.210
vasomotor symptom(2)	0.0(-3.0~3.0)	0.0(-3.0~2.0)	0.756
sleep problem(3)	1.0(-3.0~3.0)	-1.0(-6.0~2.0)	0.004
attraction(2)	0.0(-2.0~5.0)	0.0(-2.0~4.0)	0.437

**by Mann-Whitney U test.*

number of participants was small and the results could not represent general population.

Recently, the usage of oral preparation of HPE is also available in the clinics beside the subcutaneous injection. In Korea, there are few approved statistical outcomes for HPE usage, under the circumstances where the large number of people are favorable using herbal medicines, we can easily assume that the numbers of using HPE will be more than we expected. Although there are some studies which have been shown safety features about usage of HPE[18], some still make concerns about the side effects (19,20).

Conclusion

HPE injections were partially effective in QOL improvement of postmenopausal women. Large population oriented clinical trials to confirm the efficacy and the safety of HPE should be performed.

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Correlation of femoral fragments with maximum bone length of Bosnian males

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Abstract

An assessment of maximum length of fragmented femur is considered in this study. Eight separate measurements were measured: maximal length of bone, five linear segments and two transversal diameters on the upper end of bone. Sample consisted 103 femurs from skeletal remains of males, who were killed in summer 1995, mostly collected from the surface. Measurements were done according to a Rudolph Martin's method. There is correlation between some linear and transversal segments with maximal length of bone, and there is possibility for scaling of the maximal length of bone actually of the height even if there are just certain fragments. Propositions of some authors for necessarily usage of standard measurements are correct, but in the lack of such kind of fragments in practical work, it can be used non-standard measurment. All measurements are treated by linear regression statistical method with maximal length of bone as independent variable. We presented formulas for calculation of maximal length of bone from its segments with the best correlation in many different finding of fragmentary bones.

Key words: stature, fragments of femur

Introduction

The most studies dealing with estimation of stature use intact whole bones. Actually, in the forensic practice is very common the finding of fragmented and damaged bones. Most authors recommend reconstruction of maximum lenght of bone using fragments measuring. Műller's work from 1935. formulated the significance of estimation maximum bone lenght on radius, humerus and tibia measuring different linear fragments of each bone, and provided new guidelines in the studing skeletal material (Steel i McKern, 1970).

The first study on fragmented long bones, including femur, was published by Gentry Steel and Thomas McKern (1970) on the sample of 117 adult persons. Authors measured the specific segments on intact femurs, tibias and humerus and established correlations between individual segments as well as combination of segments and maximum length of bone. The problem of difficult locating of points on anterior aspect of tibias and humerus as well as one point on the posterior side of femur is emphasized. Later works were published on other long bones, on the bones of upper limbs (Rao, Gupta i Sehgal, 1989), ulna (Badkur i Nath, 1990), femur and tibia (Jacobs, 1992), tibia (Holland, 1992), and femur (Simmons i sar., 1990). Simmons (1990) brings revision of Steel's method (1970) and introduces measuring of only standard points.

The finding of thousands of human skeletons both on the surface of the earth and in the mass graves especialy the finding of isolated and damaged long bones was very common practice after the last war in Bosnia and Herzegovina. Animal activity is very important factor that affects morphology, distribution and preservation of the surface skeletal remains. Therefore it is important to use all available methods to improve the process of identification those victims.

Material and methods

103 intact femurs of adult males colected between 1996. and 2000. from the surface were measured. The bones were completely skeletonized without soft tissue and without cartilage. Only intact, undamaged bones were used. Since there is no significant differences between left and right side (Genoves, 1967), only left femurs are measured wherever possible except in the case where the left side was damaged.

Measurements:

1. Maximum length of femur (MAXD). Osteometric board. Standard measure. (Martin 1).

2. Segments of femur according to Steel (1970). Sliding caliper and flexible meter in osteometric board.
   S1- segment between most proximal points of haed and midpoint of trochanter minor
   S2- segment is lenght between midpoint of trochanter minor and point where lateral and medial supracondilar lines become paralel, on the top of popliteal surface
   S3- segment is distance between last distal point and most proximal point of fosae intercondylaris,
   S4- segment is distance between last distal point and most distal point of medial condilus
3. Non-standard segment of femur (DD). Flexible meter. The distance between midpoint of trochanter minor and most proximal point of prominence of insertion of caput mediale m. gastrocnemii.
4. Upper epiphiseal length (VHE). Sliding caliper. Upper epiphiseal length along the ossis of femoral neck is distance between most medial point of femoral head and lateral edge of bone on the intersection with ossis of femoral neck. Standard measure. (Martin 13).
5. Vertical diameter of the head (VHD). Sliding caliper. Standard measure. (Martin 18).
6. Circumference of the mid-shaft (OST). Flexible meter. Midshaft of the bone. Standard measure (Martin 7)

Standard measure according to Martin and Saller (1959).

Results

The mean value of maximum length of femur was 46,89 +/- 0,24 cm. The maximum value was 53,20 cm, and the minimum value 41,60 cm. Of five measured linear segments (S1, S2, S3, S4, DD) the longest segment was nonstandard segment of femur with midle value 31,88 +/- 0,18 cm. The shortest segment was S4, with mean value 3,88 +/- 0,30 cm. The mean value of upper epiphiseal length was 10,23 +/- 0,54 cm, and vertical diameter of the head 4,98 +/- 0,27 cm. Different combinations of consecutive linear segments can be obtained by adding up of individual values of segments. These combinations of segments can be mesured from most proximal point of the first to the most distal point of the last one. Combination of segments S1, S2 and S3 is bounded with standard points, and represents distance between most proximal point of the head and upper edge of fossae intercodylaris. The mean value of this segment was 43,01 +/- 0,23 cm. Combination of segments S2, S3 and S4, is also bounded with standard points. The mean value of this segment was 38,89 +/- 0,21 cm. Combination of segments S1 and S2, as well as S3 and S4 are not bounded with standard points. Table 1 shows mean values, minimum and maximum values of maximum length and of individual and combinations of segments.

Table 1. Statistical parameters between segments and maximum length

Segment	mean value [a]	minimum [a]	maksimum [a]	SD	SE
MAXD	46,89	41,60	53,20	2,41	0,24
S1	7,99	6,50	10,46	0,76	0,07
S2	23,34	19,83	29,40	1,82	0,18
S3	11,67	9,80	15,15	1,05	0,10
S4	3,88	30,66	40,88	0,31	0,30
S1+S2	31,34	27,17	37,40	2,08	0,20
S2+S3	34,99	3,20	4,40	1,98	0,19
S3+S4	15,55	13,30	19,35	1,13	0,11
S1+S2+S3	43,01	38,00	49,40	2,30	0,23
S2+S3+S4	38,89	34,26	44,68	2,10	0,21
VHE	10,23	9,00	12,18	0,55	0,54
VHD	4,98	4,37	5,64	0,27	0,27
DD	31,88	27,80	37,00	1,81	0,18

[a] *values in centimeters*

Table 2 shows the percentage relationship of individual segments and combinations of segments compared to maximum length of femur. The longest individual segment was segment designated as DD, with almost 68% of the total length of bone. The longest individual segment of four Steel's segments was segment S2, with almost 50% of the total length of bone. In combination with S3, represents nearly 75% of the maximum length of bone. The combinations of segments S1, S2 and S3 represents 91,72%, and the combinations of segments S2, S3, S4 nearly 83%. The mean value of upper epiphiseal length in percentage was 21,85% of the total length of bone and vertical diameter of head 10,62%.

Table 2. Percentage relationship segments compared to maximum lenght

Segment of bone	X(%)	SD	SE
S1	17,05	1,34	0,13
S2	49,77	2,40	0,23
S3	24,91	2,02	0,20
S4	8,27	0,62	0,06
S1+S2	66,81	2,09	0,21
S2+S3	74,63	1,49	0,14
S3+S4	33,18	2,09	0,21
S1+S2+S3	91,72	0,61	0,06
S2+S3+S4	82,94	1,34	0,13
VHE	21,85	1,07	0,11
VHD	10,62	0,52	0,05
DD	67,98	1,62	0,16

It is determined correlation between segments and maximum length of femur (table 3).

Table 3. Correlation between segments and maximum length of femur

Segment	r (correlation factor)	MAXD
S1	0,55	
S2	0,78	
S3	0,42	
S4	0,42	
S1+ S2	0,88	
S2+ S3	0,93	
S3+ S4	0,51	
S1+ S2 + S3	0,99	
S2+ S3 + S4	0,95	
VHE	0,55	
VHD	0,57	
DD	0,91	

The sum of values of segments S1 and S2 is in highly positive correlation with maximum length of femur (r = 0,88), (Chart 1).

The sum of values of segments S1, S2 and S3 is in highly positive correlation with maximum length of femur (r = 0,99), (Chart 2).

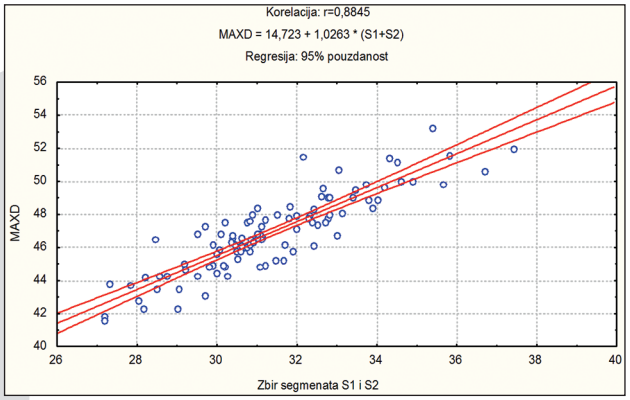

Chart 1. Correlation between S1,S2 and MAXD

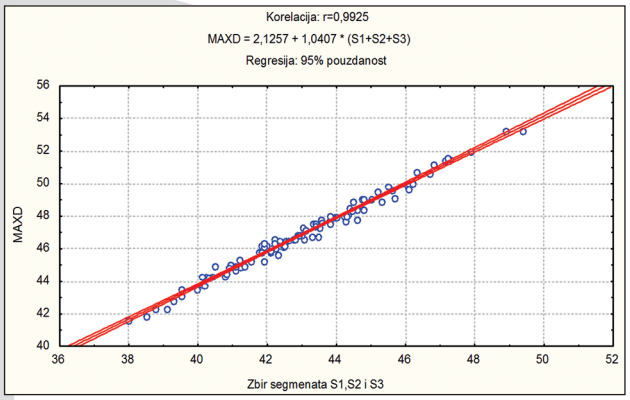

Chart 2. Correlation between S1,S2,S3 and MAXD

Vertical diameter of head and maximum length of femur are in correlation (r = 0,57), (Chart 3).

Non-standard segment of femur (DD) and maximum length of femur are in highly positive correlation (r = 0,91), (Chart 4).

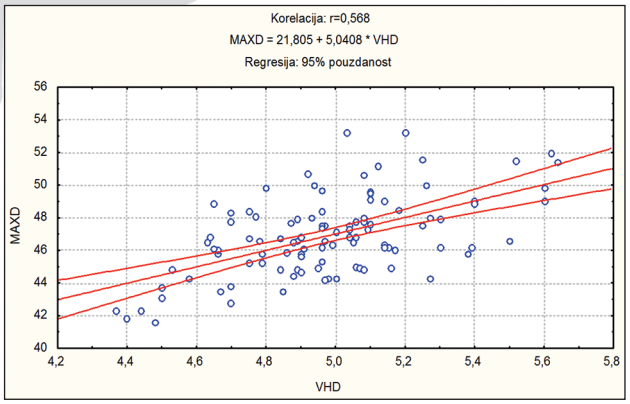

Chart 3. Correlation between VHD and MAXD

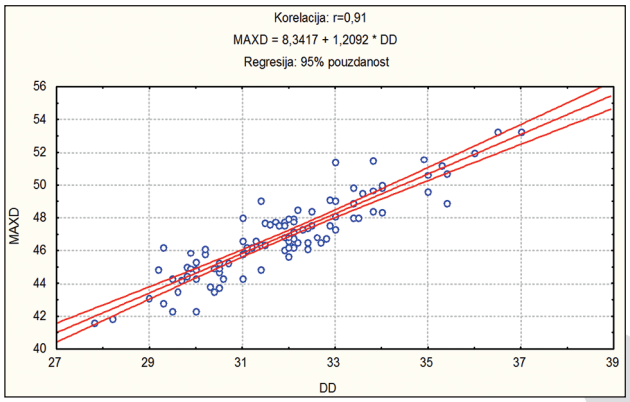

Chart 4. Correlation between DD and MAXD

Discussion

The mean value of maximum length of femur in this study is greater then those of previous studies. Hadžiselimović (1959) has determined average maximum length on 50 male femurs which is almost 2,7cm less than our average value. The period from which the sample originates is unknown. Steel and McKern (1970) have determined length of 71 male femurs from different american arheological locations from preeuropean contact and it is less than our nearly 2 cm.

Since there are not many publications in the field of physical anthropology in our geographical area it is particulary interesting to compare results of this study with previous work. Hadžiselimović (1959) has presented morphological caracteristics of long bones using different indexes in our population, but two measures were object of both studies. Those are maximum length of femur and vertical diameter of head. Table 4 shows comparative values of this and other studies. In addition to maximum length, higher values for the vertical diameter of head were obtained.

Table 5 shows mean value of percentage relationship segments and maximum length of bone. All values from this study matched with values obtained by Steel and McKern (1970), except segments S2 and S3. There is significant difference only in values of those two segments. The difference is result of using of non-standard points whose search is subject to a subjective assessment by the author. This is also confirmed by the fact that the sum of values segments S2 and S3, bounded with standard points, matched in both studies. Damaged femur on both ends, including head and neck and both condila, can be measured in this way. The sum of values segments S2 and S3 represents around 74,63% maximum length of bone and correlation is highly positive (r = 0,93). Non-standard segment of femur (DD) shows also very good correlation to maximum length of femur (r = 0,91).

Different segments can be used for measuring even longer parts of femur depending on the bone damage. If distal part of femur is damaged the three

Table 4. Comparison with other studies

Segment	This study [a]	Study 1 [a]	Study 2 [a]	Study 3 [a]
MAXD	46,89	44,90	-	44,20
S1	7,99	7,35	-	-
S2	23,34	25,19	-	-
S3	11,67	8,71	-	-
S4	3,88	3,55	-	-
S1+S2	31,34	32,55	-	-
S2+S3	34,99	33,90	-	-
S3+S4	15,55	12,27	-	-
S1+S2+S3	43,01	41,26	-	-
S2+S3+S4	38,89	33,84	-	-
VHE	10,23	-	9,91	-
VHD	4,98	-	4,83	4,81
DD	31,88	-	-	-

[a] *all values in centrimeters*
Study 1- Steel i McKern (1970)
Study 2- Simmons, Jantz i Bass (1990)
Study 3- Hadžiselimović (1959)

Table 5. Percentage relationship segments and maximum length of bone

Segment	This study			Other studies [a]		
	X(%)	SD	SE	X(%)	SD	SE
S1	17,05	1,34	0,13	16,40	1,10	-
S2	49,77	2,40	0,23	56,10	2,50	-
S3	24,91	2,02	0,20	19,40	2,50	-
S4	8,27	0,62	0,06	7,90	0,70	-
S1+S2	66,81	2,09	0,21	72,50	2,60	-
S2+S3	74,63	1,49	0,14	75,50	1,30	-
S3+S4	33,18	2,09	0,21	27,30	2,60	-
S1+S2+S3	91,72	0,61	0,06	91,90	0,70	-
S2+S3+S4	82,94	1,34	0,13	83,50	1,10	-
VHE	21,85	1,07	0,11	-	-	-
VHD	10,62	0,52	0,05	-	-	-
DD	67,98	1,62	0,16	-	-	-

[a] Steel i McKern (1970)
X(%)- mean percentage value
SD- standard deviation
SE- standard error

first segments can be measured together, which represents highly 91,72% of the total length of bone. In general, segments representing longer part of bone have better correlation to maximum length of bone. If proximal part of femur is damaged the three lower segments can be measured together, which represents 82,94% total length of bone with highly positive correlation factor (r = 0,95). The only condition is preservation of trochanter minor.

Segments S3 and S4 can be measured in the case when both ends of bone are damaged, but correlation factor is low (0,51). The measurement is not completely disabled by the damage of both ends of bone. Segments S2 and S3 can be measured if distal point of S3 segment is preserved. This segment has high correlation with maximum length of bone (r = 0,93).

Other measured values are in a lower correlation with the maximum bone length.

Conclusion

Damage of distal end of bone with clearly visible proximal point of fossa intercondylaris.

In this case the highest correlation is obtained by measuring of segments S1, S2 and S3 (r = 0,99).

The following formula can be used:

MAXD= 2,1257 + 1,0407*(S1+S2+S3) +/- 0,54 cm

Damage of distal end of bone with undetectable proximal point of fossa intercondylaris.

In this case, using only standard points, the highest correlation is obtained by measuring of vertical diameter of head (VHD) (r = 0,57).

The following formula can be used:

MAXD = 21,808 + 5,0408*VHD +/- 0,43349 cm

Alternative way is, using non-standard points, the measuring of non-standard segment (DD) (r = 0,91).

The following formula can be used:

MAXD = 8,3417 + 1,2092*DD +/- 1,479 cm

Damage of head and neck of femur.

In this case the highest correlation is obtained by measuring of segments S2, S3 and S4 (r = 0,95).

The following formula can be used:

MAXD = 4,251 + 1,096*(S2+S3+S4) +/- 1,233 cm

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Instructions for the authors

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Abstract

In this paper the instructions for preparing camera ready paper for the Journal are given. The recommended, but not limited text processor is Microsoft Word. Insert an abstract of 50-100 words, giving a brief account of the most relevant aspects of the paper. It is recommended to use up to 5 key words.

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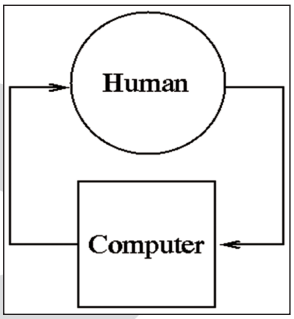

Figure 1. Text here

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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