

The Analysis of Hormone Replacement Therapy Influence on II-6 Expression and Mandible T-Score of Postmenopausal Women

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Abstract

Objective: Aim of the study was to investigate the effect of Hormone Replacement Therapy (HRT) on IL-6 gene expression and salivary concentration, bone mineral density (BMD) and T-score (the number of standard deviations below the average for a young adult at peak BMD) of mandible in menopausal women.

Materials and Methods: Study was carried out on a group of 60 women during menopause - 30 untreated (control group) and 30 treated with HRT (test group). 30 patients has undergone natural menopause and other half of women were after ovariectomy. Examination of IL-6 gene expression was conducted on peripheral blood lymphocytes (PBL) and buccal epithelial lining (BEL). Saliva samples were collected and densitometry was conducted on the mandible. To evaluate the results of densitometric examination T-score index was calculated.

Results: T-score index of the control groups reached values below (-2). T-score results for test group were higher than those in control group. HRT results in a slight decrease of the IL-6 level in saliva of the test group, compared with control group, but the differences between groups were not statistically significant. Only in groups after natural menopause treated with HRT significant increase of IL-6 gene expression was stated when compared to control groups.

Conclusions: HRT has a significant osteoprotective effect on the mandible. This local beneficial effect of HRT may be exerted, inter alia, by local decrease in salivary concentration of proinflammatory cytokine IL-6, but further research is required to clearly confirm this thesis. Results of IL-6 gene expression do not allow stating any clear thesis. It needs to be clarified if HRT has the same effect on cytokine gene expression as hormones produced by ovaries.

Keywords: IL-6; Gene expression; Cytokines; Saliva; Osteoporosis; T-score; Mandible

Introduction

Bone metabolism is a dynamic process. Both, the cancellous and compact bone are subjects of continuous internal remodeling throughout the life. The endocrine system has a major impact on the process of bone remodeling. Natural menopause or ovariectomy lead to significant decrease in the production and secretion of steroid sex hormones is observed [1,2]. Recent studies have shown that estrogen deficiency causes disorders in bone metabolism, with a predominance of resorption processes. However, the process of bone mineral density (BMD) reduction is not linear but dynamic [3].

Two types of cells play a major role in the process of connective tissue remodeling: osteoblasts (osteogenic cells) and osteoclasts (bonedestroying cells). There are two theories on the impact of estrogen on bone tissue. Estrogen affects bone tissue via cell receptors and by the indirect mechanism, but receptor mechanism has not been fully understood yet [4]. Indirect mechanism is inter alia based on the influence of the local secretion of interleukin 1 (IL-1), interleukin 6 (IL-6), Tumor Necrosis Factor alpha (TNF-alpha) and Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) on the activation of osteoclasts [5]. One of the mentioned factors which take part in the indirect regulation of the bone turnover is IL-6, produced by osteoblasts, monocytes, fibroblasts and osteoclasts. Other cells, which after specific stimulation can exert IL-6 are T-cells, B-lymphocytes, granulocytes, chondrocytes and epithelial cells. IL-6 is one of the most important factors in the acute-phase of inflammation [6]. The major biological functions of this cytokine are: differentiation and growth regulation of T-cells, differentiation of B lymphocytes into plasma cells, stimulation of IgG secretion, interaction with interleukin-3 in the process of hematopoesis, antiviral activity and stimulation of acute phase proteins production. IL-6, IL-1 and TNF are also involved in the local and systemic inflammatory response [7,8]. Many studies have confirmed the role of IL-6 in the process of bone destruction. Under the influence of osteotropic agents, such as parathyroid hormone, 1,25-dihydroxycholecalciferol and IL-1, osteoblasts secrete IL-6 which in low concentrations stimulates the osteoclast-precursor cells and induce osteoclastogenesis, whereas in high concentrations activates mature osteoclasts. Activated osteoclasts produce IL-6, which promotes the process of bone resorption [9]. IL-6 is also associated with age-related atherosclerosis, coronary heart disease and heart failure [10]. Previously there were attempts to use IL-6 as marker of bone metabolism. Researchers examined the correlation between secretion of IL-6 and processes taking part in skeleton. The IL-6 levels

Page 2 of 7

were significantly influenced by metastatic tumors occurring in bone tissue or hyperthyroidism [11,12]. Results obtained by Ramazan Sekeroglu in his work showed that there is a correlation in fluctuations between serum IL-6 level and markers of bone turnover rate [12]. Investigation of the expression of IL-6 gene in Peripheral Blood Lymphocytes (PBL) and Buccal Epithelial Lining (BEL) of postmenopausal women could show potential differences in the gene expression between postmenopausal women treated and not treated with HRT. This would allow to determine if osteoprotective effect of estrogen is exerted, inter alia, by changes in expression and metabolism of IL-6. Because of its multiple functions, research on the process of IL-6 gene expression regulation has great importance. The studies on the gene expression of IL-6 are especially needed in case of osteoporosis in which one of pathomechanisms of disease is the excessive release of proinflammatory cytokines which lead to demineralization of the skeleton.

Hormone Replacement Therapy is aimed to reduce negative effect of estrogen deficiency [13]. Supplementation with estrogen increases circulating levels of this hormone and compensates for its loss after menopause, thus relieving the symptoms of menopause. It has been shown that higher level of estrogen prevents bone loss and decreases the risk of hip fractures [14]. Studies also suggest that age-related bone loss may be the result of estrogen deficiency in men as well as postmenopausal women [15]. These studies highlight the fact that estrogen is important for the maintenance of bone mineral density in both men and women. Many studies suggested that long-term HRT, in addition, could help menopausal patients to cope with mild stress and to improve mental performances [16]. On the other hand, the treatment with HRT increases risks for venous thromboembolism and ischemic stroke, but in HRT users affected by a cardiovascular event continuation of therapy has not been found to be associated with adverse outcome [17]. Always consideration between the pros and cons of HRT is mandatory before administration of therapy. That is why the control process of osteoprotective effect is important during the evaluation of therapy advantages and disadvantages. This is also the reason for the search of new, simple and non-invasive methods of evaluation of processes taking part in bone tissue.

The aim of the study was to investigate the effect of Hormone Replacement Therapy (HRT) on IL-6 gene expression and salivary concentration, Bone Mineral Density (BMD) and T-score (the number of standard deviations below the average for a young adult at peak BMD) of mandible of menopausal women. Analysis of possibility of use of saliva for evaluation of IL-6 levels fluctuations could significantly improve testing procedures concerning IL-6. Saliva is easy to obtain and the process of collecting of saliva is simple and non invasive for patient. Analysis of BEL IL-6 gene expression, concentration of IL-6 in saliva and evaluation of mandible BMD may show the local benefits of hormone replacement therapy.

Materials and Methods

The research was conducted in a group of 30 menopausal women undergoing HRT for 6 months (age range 49 – 59 years mean age 53.0 years) (study group). Women from study group were supplemented with combination HRT (estrogen and progesterone in combination). Patients received Femoston in tablets (2mg of Estradiol hemihydrate and 10 mg of dydrogesterone). Estrogen was taken on a continuous basis. Patients received progesterone for the last 14 days of each course (course lasted 4 weeks). The control group consisted of 30 postmenopausal women, at least 12 months after the last menstruation (age range 53 - 59 years, mean age - 55.4 years). Patients in the control group have never been receiving HRT. Patients were treated in the out-patients gynecological clinic of Public Hospital No. 4 in Lublin (Poland). Control and research groups were divided into four subgroups: M - a group of menopausal women, OV - a group of women after surgical removal of ovaries, OV + HRT - a group of women after surgical removal of ovaries using HRT, M + HRT - a group of menopausal women using HRT. Patients after ovariectomy (mean age 54,2 years) underwent surgery at least 36 months before the study was conducted. Surgeries were performed as treatment of diseases of reproductive system. In case of 5 of patients from group OV and 4 form group OV + HRT the indications for ovariectomy were malignant tumors. In case of patients with malignant tumors no metastasis had been discovered. None of patients was receiving neither chemo- nor radiotherapy at least for 2 years before study. Testing procedures received approval of Local Ethics Committee in Lublin. The study was carried out in accordance with the ethical principles contained in the Declaration of Helsinki. All patients gave their consent to the examination and research protocol.

The questions in anamnesis chart included: age, occupation, socioeconomic status, and date of last menstrual period, duration of HRT, addictions, physical activity, medications and surgeries. After examination, unstimulated saliva was collected from women in the fasting state. Saliva samples were collected in morning hours (7-9 a.m.). Chewing gum was prohibited for at least 2 hours before the test. Patients before collection of saliva were asked to rinse mouth with distilled water and relax for couple minutes, in order to obtain unstimulated saliva. Then the patients were asked to lean their head forward and keep their mouth open for 5 minutes in order to allow saliva drain into the testing tube. At the end of collection process, women were asked to spit remaining saliva into the testing tube. Nonstimulated saliva is produced in 70% by submandibular glands, which produce serous-mucous secretion [18]. Mixed saliva should be used for diagnostic purposes, because it contains components of blood serum [19]. After centrifugation of the material, obtained supernatant (saliva) was stored until biochemical tests at temperature -70°C. The concentration of cytokine IL-6 in saliva was determined by Enzyme-Linked Immunosorbent Assay (ELISA), using Kit from BD Biosciences Pharmingen performed following the procedure specified by the manufacturer.

Epithelial lining of buccal mucosa was collected and suspended in 5 ml of 0.9% saline and centrifuged for 15 minutes at speed of 3000 rpm in order to separate the epithelium. Obtained buccal epithelium and newly drawn, uncentrifuged blood collected in an EDTA tube were used for analysis of gene expression: a control GAPDH gene and IL-6 gene. Total cellular RNA isolation was performed using Tri Reagent Sigma reagent (method modified by Chomczynski and Sacchii) according to the procedure provided by the manufacturer [20]. Following complete dissociation of the nucleoprotein complex (5 minutes at room temperature), 0.2 ml of chloroform was added, shaken vigorously, incubated for 15 minutes, also at room temperature and centrifuged at a 12,000 rpm for 20 minutes at 4°C degrees. Lysate was divided into three phases: the organic phase containing protein, interphase containing DNA and colorless upper aqueous phase containing RNA. After transferring the aqueous phase to new Eppendorf test tubes, 0.5 ml of isopropanol was added. The samples were left for 10 minutes at room temperature, and then centrifuged at 12,000 rpm for 10 minutes at 4°C. After removing the supernatant, RNA residue was washed with 75% ethanol, centrifuged (7500 rpm) for 5 minutes, dried and then dissolved in H2O. Obtained RNA was

used to prepare cDNA. RNA isolation from blood (1 ml) was preceded by the lysis of erythrocytes in a buffer composed of NH4CL 0.8M; KHCO3 0.05M; EDTA 0.01M. After 30 minutes of incubation at 4°C, blood was centrifuged (12,000 rpm) for 20 minutes at 4°C. The supernatant was discarded, and RNA was isolated from the sediment according to the method described above. cDNA synthesis was performed using a reagent kit: High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems), in accordance with the procedure recommended by the manufacturer. 2 µg of total RNA were used per 20-µL reaction. The 2 x RT master mix was prepared using the kit components before preparing the reaction plate. During the procedure Kit with RNase Inhibitor and Multi Scribe™ Reverse Transcriptase was used. 10 µL of 2 x RT master mix and 10 µL of RNA sample were pipetted into each well of a 96-well reaction plate or individual tube. The plate was centrifuged to spin down the contents and to eliminate any air bubbles. Reverse Transcription was performer in thermal cycler. Step 1 of reaction lasted for 10 min in temperature 25°C. Second face of process lasted for 120 min in temperature 37°C. Step 3 of reaction lasted for 5 min in temperature 85°C. cDNA Reverse Transcription Reactions were stored at temperature of -15 to -25°C. cDNA was used for "real-time"-PCR in order to determine the level of GAPDH (control gene) and IL-6 gene expression. TaqMan[®] glyceraldehyde-3-phosphate dehydrogenase Control Reagents [Human](Hs03929097_g1) and TaqMan^{*} IL6 Gene Expression Assay (Hs99999032_m1) were used.

The study of mandible Bone Mineral Density (BMD) was performed in the Densitometric Laboratory of Institute of Agricultural Medicine in Lublin, by means of the DPX-A (General Electric Healthcare Technologies Lunar) Prodigy Advance and absorptiometry of X-rays beams of two energies [21]. DPX-A is a convenient and safe method of measuring bone density, where the amount of ionizing radiation needed to perform the test is less than 3 mm, representing 0,1 radiation used to perform a routine chest X-ray [22]. Scan time lasted for 30 seconds. The area of interest was determined by analysis with software en Core (version 10.50). Images of the mandible were scanned and area of angle of the mandible was marked and the average BMD of the area was calculated Bone density was specified in g/cm2. To evaluate the results of densitometric examination T-score index was calculated. T-score is the ratio of BMD of the examined patient to Page 3 of 7

the average bone density of young people [23]. T-score values characterizing the bone quality are defined as:

- 1. healthy bone T-score higher than (-1),
- 2. osteopeny a T-score between (-1) to (-2.5),
- 3. osteoporosis T-score less than (-2.5).

In order to calculate T-score of the mandible, the reference mean (M) and standard deviation (SD) of the mandible BMD were calculated. 20 healthy women (age range 19 to 21 years, mean age 20,5 years) were selected for reference group and the following results for the mandible bone density were obtained: reference M = 1.2889 and reference SD = 0.3877. Knowing the M and SD of the reference group of women allowed to calculate T-scores for control and research groups [T-score = (mandible BMD – M) / SD].

Results were statistically analyzed with use of program Statistica. The arithmetic M and SD were calculated. The significance of differences between groups is based on confidence intervals [LSD test] determined from the analysis of variance (ANOVA). The unpaired T-test was used in order to determine whether results differed significantly. The risk of error of inference of the study is 5%, which means that the results were significant, if the 'p' was equal or less than 0,05.

Results

Basic data about the patients enrolled for the study are presented in Table 1. Body Mass Index was calculated for each patient. Patients qualified for the study had BMI values between 27-29 (values classified as overweight, but not obese). Patients qualified for the study did not suffer from any severe general diseases, had no addictions and had not been taking medications continuously. Patients qualified for the study were non-smokers. Clinical examination was focused on: the presence of any pathological lesions of mucosa, dental status, used dentures and oral hygiene. Patients qualified for further tests did not have any acute inflammations of oral mucosa and the depth of periodontal sockets was lower than 5 mm. Patients qualified for the study did not need any extractions of teeth and had average or good oral hygiene. Women qualified for the study did not use any removable dentures. No previous fractures of the mandible were noted.

Research group	Sample number	Age [Years] (M ± SD)	Control group	Sample number	Age [Years] (M ± SD)	Statistically significant differences between results in control and research group (p<0.05)
	15	53,27 ± 3,13		15	55,20 ± 2,21	No
	15	52,73 ± 2,81		15	55,60 ± 2,10	No
		Time from last menstrual period [Months] (M ± SD)			Time from last menstrual period [Months] (M ± SD)	
	15	43,3 ± 3,8		15	41,5 ± 3,9	No
	15	41,5 ± 5,1		15	42,7 ± 4,1	No
		Duration of HRT [Months]			Duration of HRT [Months]	

Page 4 of 7

	(M ± SD)		(M ± SD)	
15	17,0 ± 3,8	15	17,6 ± 3,6	No
15	17,6 ± 4,1	15	16,8 ± 3,5	No
	Body Mass Index (M ± SD)		Body Mass Index (M ± SD)	
15	27,73 ± 0,70	15	27,87 ± 0,83	No
15	27,87 ± 0,83	15	27,80 ± 0,77	No

Table 1: Basic data about patients qualified for the study

Treatment with HRT in groups (M + HRT) and (OV + HRT) resulted in the increase of the mandible density, compared to the BMD in groups (M) and (OV). Differences in BMD between test and control groups were distinct, but only the difference between groups (M + HRT) and (M) was high enough to be statistically significant (1,80 g/cm2 in group M+HRT, compared to 0,50 g/cm2 in group M) (Table 2).

T-score index in groups (M), (OV) and (OV + HRT) reached levels below -1, which means that in these patients osteopenic changes in bone tissue were observed. (Table 2) Values of T-score were significantly higher in test groups when compared to values in control groups, but the difference was high enough to be statistically significant only between groups (M + HRT) and (M) (1,31 compared to -2,03).

In groups treated with HRT the concentration of IL-6 in saliva was lower than in groups (M) and (OV), but the difference was not high enough to be statistically significant (Table 3).

In groups after natural menopause treated with HRT significant increase of IL-6 gene expression was stated in epithelial lining of the cheek and peripheral blood lymphocytes, when compared to control groups.

Differences in gene expression between groups after ovariectomy were not high enough to be statistically significant (Table 4).

Test group	Sample quantity	BMD of mandible g/cm2 (M ± SD)	Control group	Sample quantity	BMD of mandible g/cm2 (M ± SD)	Statistically significant differences between results in control and test group (p<0.05)
M+HRT	15	1,80 ± 0,73	м	15	0,50 ± 0,33	Yes
OV+HRT	15	0,71 ± 0,33	OV	15	0,45 ± 0,29	No
		T-score for the mandible (M ± SD)			T-score for the mandible (M ± SD)	
M+HRT	15	1,31 ± 1,88	м	15	-2,03 ± 0,86	Yes
OV+HRT	15	-1,48 ± 0,85	OV	15	-2,17 ± 0,75	No

Table 2: BMD and T-score levels in research and control groups

Test group	Sample quantity	Level of IL-6 in saliva [pg/ml] (M ± SD)	Control group	Sample quantity	Level of IL-6 in saliva [pg/ml] (M ± SD)	Statistically significant differences between results in control and test group (p<0.05)
M+HRT	15	17,59 ± 4,28	м	15	22,12 ± 8,24	No
OV+HRT	15	21,05 ± 11,10	OV	15	26,89 ± 8,39	No

 Table 3: Level of IL-6 in saliva

		expression level in the epithelial lining of the cheek (M ± SD)			expression level in the epithelial lining of the cheek (M ± SD)	differences between results in control and research group (p<0.05)
M+HRT	15	1,36 ± 0,68	М	15	0,27 ± 0,19	Yes
OV+HRT	15	0,32 ± 0,09	OV	15	0,44 ± 0,35	No
		Ratios of IL-6 to control GAPDH gene expression level in peripheral blood lymphocytes (M ± SD)			Ratios of IL-6 to control GAPDH gene expression level in peripheral blood lymphocytes (M ± SD)	
M+HRT	15	8,72 ± 5,04	М	15	1,39 ± 1,28	Yes
OV+HRT	15	2,93 ± 2,69	OV	15	1,29 ± 1,02	No

Table 4: Ratios of IL-6 gene to control GAPDH gene relative expression in epithelial lining of the cheek and peripheral blood lymphocytes

Discussion

Administration of HRT in females with reduced secretion of sex hormones is aimed to prevent or reduce the climacteric symptoms resulting from the cessation of ovarian endocrine activity. One of these symptoms is osteoporosis. Osteoprotective effect of HRT on vertebral column or femur has been confirmed by many authors [24]. The area of interest of present study was the local effect of HRT on the mandible. The study was designed to determine whether the use of HRT has a significant influence on reduction of the IL-6 concentration in menopausal women and clarify if the potential osteoprotective effect of HRT on mandible may be expressed partially through the influence of sex hormones on this proinflammatory cytokine. Results of the previously conducted studies are not fully consistent [25]. The mineral density of mandible is influenced not only by systemic but also a number of local factors. Severity of periodontal disease, periapical inflammations or osteolytic pathological changes has a significant effect on the mineral density of the jaw bones [26]. Odontogenic and periodontal changes affect significantly the area of the corpus of mandible. In order to eliminate interference caused by local factors in present study the angle of the mandible was used for the measurement of the BMD. None of patients qualified for the examination had an impacted third molar or pathological lesion in the area of the angle of mandible. Examined patients had no pathological osteolytic changes in the bones of stoamatognathic system and no acute inflammations of oral mucosa were discovered. Patients with severe chronic periodontitis were excluded from the study. Both, postmenopausal and ovariectomized women, treated with HRT had a higher BMD as well as T-score of the angle of the mandible compared to women from control groups, however only between groups (M +HRT) and (M) the differences were high enough to be statistically significant. The lower results of BMD in group (OV+HRT) could be the result of oncological past of patients. Although only in case of 4 patients the reason for surgery and postsurgical chemotherapy were malignant tumors. In case of group (OV) the number of patients with malignant tumor was higher (5 patients), but differences in BMD between groups (OV) and (M) were not statistically significant. These results are consistent with data obtained by Drozdzowska and associatates. Authors of that study proved that in edentulous menopausal patients not treated with HRT mandibular bone loss is much higher than in other skeletal sites [27]. Other authors confirm the beneficial effect of sex hormones supplementation not only on the

mandibular bone tissue, but on the whole stomatognathic system. Results obtained in present study confirm that HRT has osteoprotective effect on mineral density of the mandible. Patients treated with HRT have less severe periodontal disease and a greater number of teeth [28]. What is more, the positive effect on bone remodeling prevents patients from pathological fractures which can occur in patients after menopause.

The concentration of IL-6 in body fluids is influenced not only by local but also systemic factors, such as hormone levels, general diseases or neoplasms. Study by Saheb Jamee and associates indicated a relevant connection between oral squamous cell carcinoma and elevated concentration of IL-6 in unstimulated saliva [29]. Straub and colleagues examined the level of IL-6 in blood serum of postmenopausal women. Obtained results clearly indicated the significant HRT-related inhibition of IL-6 secretion [30]. Authors suggested that IL-6 inhibition could be an important element of favorable effects of. The level of IL-6 in the saliva of the study groups (M + HRT) and (O + HRT) has been lower than in control groups (M) and (O). The differences between groups were noticeable but not high enough to be statistically significant. Patients in control and test groups were matched in term of age and health. All of the patients were no-smokers, which is vital for the content of cytokines in saliva. Buduneli and associatates examined the level of OPG/RANKL ratio in saliva of smokers [31]. Smokers exhibited higher RANKL and lower OPG content in saliva than patients who did not smoke. Significant deviations from the mean values of IL-6 content in saliva obtained in present study could be the result of the difference in the level of severity of periodontal disease between the patients in each group. Broader study group would allow excluding outmost results which would allow obtaining a clearer conclusions. The results show, however, a trend which may indicate that HRT may reduce the local concentration of IL-6 in saliva which can positively affect the bone tissue of the mandible. Reduction of local proinflammatory factor concentration seems to be one of important beneficial effects of HRT.

IL-6 is produced not only by cells which are part of immune system, but also by other types of cells, such as osteoblasts, adipocytes or epithelial cells. The expression of IL-6 is stimulated by various signals such as proinflammatory cytokines, toxins or pathogens. Various stimuli have effect on the transcription of IL-6 genes via different mechanisms. Overexpression of IL-6 is present in a number of diseases, including neoplasms, rheumatoid arthritis, Castleman's

disease or Alzheimer disease. Regulation of gene expression is a complex and multifactorial process. Gene expression depends on the cell type, the development stage of the organism, the metabolic / physiological state of the cell. For about three decades, attempts have been made to identify the molecular determinants of genes directly or indirectly affecting bone homeostasis [32]. Among the genes which seem to play important role in the process of demineralization of skeleton is gene encoding IL-6 [33]. Estrogens can modify the function of immune system. Multiple studies proved that estrogens regulate the cytokine gene expression in different cell types, either directly through the receptor or indirectly by interaction between estrogen receptor and other transcription factors, such as NF-kB or AP-1 [34]. Study conducted by Hirano and associates has shown that estrogen directly through its receptor enhances NF-kB activity of lymphocytes [35]. Results of present research showed that gene expression level in the epithelial lining of cheek and peripheral blood lymphocytes were significantly higher in women treated with HRT who have undergone natural menopause than in other groups. A significant correlation between the level of gene expression in epithelial lining and blood lymphocytes was stated (Pearson's Correlation= 0,7269). Rachoń and associates, who in their work studied the influence of estrogen deficiency and expression of IL-6, enrolled in their study healthy postmenopausal women with clinical symptoms of estrogen deficiency. Results obtained by authors indicate that estrogen deprivation after menopause leads to stimulation of IL-6 expression by peripheral blood mononuclear cells [36]. Multiple studies on the effect of HRT administration on the serum level of IL-6 proved that estrogen supplementation leads to decrease in IL-6 release. The issue which needs to be cleared is if hormone supplementation has the same effect on cytokine gene expression as natural autogenic hormones produced by ovaries. What is more, further studies should be aim to clarify whether natural menopause shows the same mechanism of influence on gene transcription as estrogen deficiency caused by the cessation of ovaries' function.

Conclusions

HRT has a significant positive influence on bone tissue of the mandible. Menopausal patients treated with sex hormones have higher levels of bone mineral density and T-score values of mandible. Results obtained in present study allow raising a thesis that HRT leads to a slight decrease in salivary concentration of IL-6. Lower concentration of proinflammatory factor in saliva may have a local osteoprotective effect on the mandible. This knowledge may prove to be relevant in the treatment of patients, who are vulnerable to osteoporotic changes, including osteolytic changes in the bones of stomatognathic system. Results of present research showed that gene expression level in the epithelial lining of the cheek and peripheral blood lymphocytes were significantly higher in women treated with HRT who have undergone natural menopause. It needs to be cleared if estrogen supplementation has the same effect on cytokine gene expression as natural hormones produced by ovaries. It seems reasonable to conduct further research on a broader group of patients in order to clearly support trends shown in present study.

Declaration of Interest

Authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported. This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector. All authors conceived the idea for the study and contributed to the design and planning of the research. All authors were involved in data collection and analyzed the data. All authors contributed to the process of writing the manuscript. All authors edited and approved the final version of the manuscript.

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Page 6 of 7

Page 7 of 7

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