MULTIDIRECTIONAL ASSESSMENT OF BONE STRUCTURE INCLUDING RADIOISOTOPIC ANALYSIS IN PERIMENOPAUSAL WOMEN

W. Tryniszewski^{1,*}, I. Raciborska¹, Z. Maziarz¹, M. Nowak¹, M. Radek²

Lodz University of Medicine - ¹Department of Radiological and Isotopic Diagnosis and Therapy, ²Department of Neurosurgery and Peripheral Nerves Surgery, Lodz, Poland

Abstract

Objective. In postmenopausal period, changes in bone turnover markers (BTM), vitamin D3, cytokines and parathyroid hormone (PTH) are frequently observed. The study was to assess bone mineral density (BMD) and bone metabolism index (IBM) in the perimenopausal women.

Design years: 2013-2014.

Subjects and Methods. One hundred and thirteen women were divided into four groups: group I (35 not menstruating 50 - 60 years old with osteoporosis), II (23 not menstruating 50 - 60 years old without osteoporosis), III (30 menstruating 40 - 49 years old with osteoporosis), IV (25 menstruating 40 - 49 years old without osteoporosis). The following parameters were measured: IL-1 β , IL-6, TNF- α , hormone oestradiol (E2), PTH, FSH, TSH, calcium (Ca²⁺), phosphates (P), alkaline phosphatase (bALP), C-terminal telopeptide of type I collagen alpha 1 chain (α 1CTX), osteocalcin (OC), BMD, IBM.

Results. IBM and BMD were significantly lower in premenopausal than in postmenopausal women. The concentration of OC, CTX, 25OH D3 and PTH levels differed significantly between group I vs. II, group I vs. III and group II vs. IV.

Conclusions. The levels of BTM, D3, PTH differed significantly between groups. This study demonstrated that bone metabolism depended mainly on processes related with menopause state and changes in D3, PTH and cytokines levels.

Key words: bone metabolism, bone scintigraphy, cytokines, menopause, osteoporosis.

INTRODUCTION

Bone metabolism is characterized by resorption and new bone formation processes. Premenopausal protective effect of oestradiol results in the stabilization of pro-inflammatory cytokines at low levels. In menopausal period, the decline in oestrogen concentration is accompanied by changes in the level

of cytokines, vitamin 25OH D3 (D3) and parathyroid hormone (PTH) leading to the exacerbation of osteoporosis. The aforementioned mechanisms are responsible for up to 50 % of cases of this disease (1).

Apart from densitometric examination, multidirectional assessment of different bone metabolism indexes and complex interpretation of obtained results are indicated to be performed for a full evaluation of bone turnover in perimenopausal period (2-5). For this purpose, the analysis of crucial parameters of premenopausal period, such as: bone turnover markers, vitamin D3, hormones and other laboratory tests in serum as well as densitometric examination and radioisotopic assessment of bone metabolism index were performed. Scintigraphy allows for the visualization of osteoporotic changes associated with 8 - 15% decalcification, while in radiographic examination, at least 30% changes are seen.

Dynamic bone scintigraphy with the use of developed method and program enables the determination of the index of bone metabolism (IBM) (6). Within the last ten years, the use of IBM has been presented in our articles (5-8).

In this study, numerous research methods were used to assess bone quality in women in premenopausal and postmenopausal period. Premenopausal period referred to regularly menstruating 40 - 49 years old women, in whom symptoms including hot flushes, hyperhidrosis, sleeping disorders and mood swings worsened with time. Postmenopausal period referred to women not menstruating for at least 5 years.

The assessment of relationships between obtained results may provide new diagnostic information concerning this period of women's life.

The objective of this study was to assess bone metabolism in the perimenopausal women on the basis of cytokines, hormones, bone turnover markers and biochemical parameters concentrations as well

*Correspondence to: Wiesław Tryniszewski PhD, Medical University, Department of Radiological and Isotopic Diagnosis and Therapy, Zeromskiego 113, Lodz, 90-549, Poland, E-mail: wieslaw.tryniszewski@umed.lodz.pl

as the results of imaging tests and the analysis of the correlations between these parameters.

MATERIALS AND METHODS

Following the analysis of several hundred questionnaires concerning health state, dietary habits, addictions, used medications and surgical procedures, 113 women were enrolled into this study and divided into age group: 40-49 and 50-60 years in premenopausal and postmenopausal period respectively.

The exclusion criteria were:

1. Chronic administration of bone metabolism affecting agents: bisphosphonates, Strontium ranelate, sodium fluoride, denosumab, selective oestrogen receptor modulators, glucocorticosteroids, anti-epileptic drugs, aromatase inhibitors, denosumab, GnRH analogues and chemotherapy.

2. Frequent intake of antipyretics.

3. The usage of hormonal replacement therapy or the usage of oestrogen therapy in the postmenopausal period.

4. Endocrine disorders, significantly affecting bone resorption processes, such as: hyperparathyroidism, active hyperthyroidism, hyperprolactinaemia and hypogonadism.

5. Chronic renal failure (glomerular filtration rate < 35 ml/min).

6. Malabsorption.

7. Diagnosed cancer, malignant tumour or process.

8. Chronic systemic disease that could have significantly affected bone metabolism (e.g. gout and rheumatic diseases, etc.)

9. Disorders of the skeletal system which make it impossible to perform planned imaging tests e.g. scoliosis and a history of neurosurgical spine treatments.

10. No written consent to participate in the study.

All patients were informed about the purpose and methods used in this study and they signed informed consent to participate in it. Women enrolled in this study declared their normal daily activity without any additional regular exercise. They all took care of proper nutrition with the average consumption of 2000 calories a day.

Women enrolled into the study were divided on the basis of age, premenopausal or postmenopausal period and bone densitometry test results into the four following groups:

Group I – N=35; 50 – 60 years old women, not menstruating for at least 5 years, with diagnosed osteoporosis (T-score $\leq$ -2.5 SD),

Group II – N=23; 50 – 60 years old women, not menstruating for at least 5 years, with osteopenia or normal BMD (T-score $>$ -2.5 SD),

Group III – N=30; menstruating 40 - 49 years women with diagnosed osteoporosis (Z-score $\leq$ -2 SD) and with worsening menopausal symptoms,

Group IV – N=25; menstruating 40 - 49 years old women with osteopenia or normal BMD (Z-score $>$ -2 SD) and with worsening menopausal symptoms.

In this study we carried out: *Anthropometric tests*: age, height, weight and BMI.

Serum blood parameters:

Blood samples from all study participants were drawn in the morning after an overnight fasting. In case of premenopausal women, blood samples were collected during luteal phase.

Cytokines: for the analysis of cytokines levels, ELISA (Enzyme-Linked Immunosorbent Assay) tests of interleukin IL-1 β , interleukin IL-6, tumour necrosis factor α (TNF- α) using Diaclone kits were performed.

Markers of bone turnover: the assessment of bone turnover was carried out with the use of serum bone formation marker - bone GLA protein osteocalcin (OC; normal range 2 - 15 ng/mL), and bone resorption marker - C-terminal telopeptide of type I collagen alpha 1 chain (α 1CTX; normal range in postmenopausal women: 0.142 $\div$ 1.351 ng/mL and 0.112 $\div$ 0.738 ng/mL in premenopausal women).

Hormones and vitamin D3: the concentrations of oestradiol (E2), follicle-stimulating hormone (FSH), parathyroid hormone (PTH), thyroid-stimulating hormone (TSH) and 25OH vitamin D3 were measured with ELFA (Enzyme Linked Fluorescent Assay) technique with the use of bioMerinex reagents.

Biochemical tests in serum: level of ionized calcium (Ca²⁺), bone fraction of alkaline phosphatase (bALP) and phosphates (P) were measured. Tests were performed with the use of reagents from Bor-Pol (Gliwice) and results were obtained using A15 analyser.

Densitometric examination

DXA technique (dual-energy X-ray absorptiometry) was applied in this study. Lumbar spine and femoral neck bone mineral density (BMD) and T-score were evaluated on a Norland XR46 machine (Norland Corp. Fort Atkinson, WI, USA). Osteoporosis was diagnosed when T-score was $\leq$ - 2.5.

Radioisotopic examination

Radioisotope analysis of bone system was performed with Philips Brightview XCT gamma camera (Philips, Guildford, UK) using own method and a developed program - BONS. The study involved dynamic acquisition of femoral bone performed in rear projection after the injection of radiopharmaceutical (Tc99mMDP) with an activity of 11 MBq/kg of body mass as well as static acquisitions performed after 180 min. from radiopharmaceutical injection.

Following the analysis of data obtained using the developed BONS program and static and dynamic bone scintigrams, radioisotopic index of bone metabolism (IBM) was calculated from the formula:

$$IBM = \frac{(G_{tot} - G_{roi}) \times 361.2 \times \ln(2)}{G_{tot} \times \text{EXP}(T_0 - T_1)}$$

where:

IBM - radioisotopic index of bone metabolism

G_{tot} - number of counts per one matrix pixel in the study of the whole body;

G_{roi} - number of counts per one matrix pixel in the selected area of interest (ROI);

T_0 - time of dynamic phase beginning;

T_1 - time of static phase beginning.

IBM is dimensionless and its normal range for healthy men is $84.05 \leq IBM \leq 105.0$. The method of determination of radioisotopic index of bone metabolism (IBM) and the results obtained in studies of healthy subjects and patients with various diseases have been published in the literature (4-6).

Statistical Analysis

All calculations were performed with the use of Microsoft Excel and STATISTICA 8 PL software. Mean value, median, highest and lowest values and standard deviation were computed. The hypothesis concerning normal distribution of individual datasets was verified with Shapiro-Wilk test. Correlation

between the results of bone imaging and biochemical tests was calculated. Statistical differences between the results of different groups of women were verified. The analysis of results was made using 95% confidence interval.

The study was approved by the Bioethics Committee of Medical University (protocol - RNN/65/12/KB dated January 17. 2012).

RESULTS

Anthropometric tests

The results of anthropometric examinations were summarized in Table 1.

Groups were found to be homogeneous in terms of age. There were no statistically significant differences between the group I vs. the group II and the group III vs. the group IV.

The results of hormonal and biochemical tests are presented in Table 2.

Concentrations of analysed cytokines, in spite of differences observed between groups, were all within the normal range. Obtained results indicated that levels of cytokines: IL- β , IL-6 and TNF- α increased and bone metabolism decreased along with age. The concentration of vitamin D3 differed between groups but it was still low in all of them. D3 levels below the lower limit of normal were found in 93% women in group I, in 60% in group III, in 45% in group II and in 18% of women in group IV. TSH levels in all groups were within the normal range. However, in osteoporotic women, TSH was slightly higher than in groups without osteoporosis. PTH concentration, despite being different between analysed groups, was also within the normal range. However, in groups comprising women with osteoporosis, PTH values were significantly higher than in groups without osteoporosis. Normal FSH values are different in pre- and post-menopausal period. These differences are presented in details in Table 2. In all studied groups, the concentration of FSH was within normal range.

Table 1. The results of anthropometric examinations

	Age [years]	Weight [kg]	Height [cm]	BMI [kg/m ²]
Group I N = 35				
mean	56.20 $\pm$ 3.78	65.31 $\pm$ 2.94	161.51 $\pm$ 3.78	25.04 $\pm$ 0.94
Group II N = 23				
mean	53.91 $\pm$ 3.09	63.26 $\pm$ 3.48	160.13 $\pm$ 4.10	24.67 $\pm$ 0.86
Group III N = 30				
mean	43.87 $\pm$ 2.80	65.03 $\pm$ 4.91	161.77 $\pm$ 4.59	24.83 $\pm$ 1.19
Group IV N = 25				
mean	43.12 $\pm$ 2.55	64.76 $\pm$ 3.41	161.80 $\pm$ 4.14	24.76 $\pm$ 1.47

Table 2. Results of hormonal and biochemical analyses in the serum

Parameter	Normal range	Group I	Group II	Group III	Group IV
IL-1 β	15÷500 pg/mL	317.25±31.61	241.73±26.97	261.95±42.47	213.60±24.53
IL-6	2 ÷200 pg/mL	148.41±25.91	103.26±15.44	118.14±30.83	89.55±16.24
TNF- α	0 ÷ 800 pg/mL	686.15±54.85	527.72±26.99	462.55±42.38	290.17±30.16
D3	30 ÷ 100 ng/mL	17.13±6.86	27.23±6.43	23.76±2.98	30.19±4.09
TSH	0.3 ÷ 4.0 mU/L	3.42±0.79	2.72±0.52	3.23±0.62	2.59±0.58
PTH	15 ÷ 68pg/mL	62.87±2.11	39.76±3.07	46.76±2.85	38.30±3.76
FSH _{luteal}	1.5 ÷ 7.0mIU/mL	—	—	6.13±0.81	5.48±0.97
FSH _{menop.}	17 ÷ 95mIU/mL	76.13±6.31	72.13±8.25	—	—
E2 _{luteal}	43 ÷ 214 mLU/mL	—	—	96.9±22.56	104.33±15.05
E2 _{menop.}	10 ÷ 56mIU/mL	5.50±4.33	6.32±9.74	—	—
P	2.6 ÷ 4.8 nmol/L	2.98±0.58	3.47±0.25	3.81±0.36	4.14±0.39
bALP	98 ÷ 279 U/L	113.04±10.15	148.86±25.20	184.26±16.43	228.25±32.88
Ca ²⁺	0.98÷1.13mmol/L	1.00±0.12	1.03±0.06	0.99±0.15	1.08±0.04
hOST	2.0 ÷ 15.0ng/mL	2.505±0.639	4.676±0.364	3.830±0.644	7.839±0.815
CrossLaps	0.142÷1.351ng/mL	1.198±0.184	0.900±0.030	0.762±0.038	0.588±0.112

Table 3. The results of densitometric examination Groups I and II

	Lumbar spine [L2÷L4]		Femoral neck	
	BMD [g/cm ²]	T-score	BMD [g/cm ²]	T-score
Group I N = 35				
mean	0.6421±0.0423	-2.837±0.2556	0.6799±0.0510	-2.713±0.3177
Group II N = 23				
mean	1.0218±0.0527	-0.5370±0.2757	0.9032±0.0324	-0.737±0.2579

Table 4. The results of densitometric examination Groups III and IV

	Lumbar spine [L2÷L4]		Femoral neck	
	BMD [g/cm ²]	Z-score	BMD [g/cm ²]	T-score
Group III N = 30				
mean	0.6482±0.0387	-2.312±0.3098	0.6928±0.0364	-2.622±0.1507
Group IV N = 25				
mean	1.0401±0.0181	-0.6178±0.1087	0.9595±0.0185	-0.2564±0.0509

The concentration of E2 in all women in group I and in 81% in group II was below the lower limit of normal. In groups III and IV, E2 levels were within normal range for this age range. In 14% of women in group I, the results of bALP were below the lower limit of normal, while P and Ca²⁺ were below normal range in 17% and 19% of women, respectively. Also in 11% of women in III group, levels of Ca²⁺ were below the lower limit of normal. In other groups, in all women, concentrations of bALP, P and Ca²⁺ were within the limit of normal. Levels of biochemical markers of bone turnover, despite being different between analysed groups, were also within normal range. hOST concentrations were associated with age and menopausal state as well as with the level of IBM and BMD. The lowest hOST values were observed in group I, while the highest in group IV. In groups of women with normal BMD and IBM, hOST values were greater than in groups with osteoporosis.

Concentrations of CrossLaps were highest in group I. High levels of CrossLaps could be the

result of enhanced activity of osteoclasts stimulated by increasing concentration of pro-inflammatory cytokines which accelerate bone resorption and reduce bone metabolism.

The results of densitometric examinations of lumbar spine and femoral neck are summarized in Tables 3 and 4.

The distribution of all BMD, T-score and Z-score values at lumbar spine and femoral neck in all groups were in normal range. In all studied women, a strong positive correlation between results of lumbar spine BMD and femoral neck BMD was observed.

A strong positive relationship between BMDLumbar spine and BMDFemoral neck confirms the absence of degenerative changes within lumbar spine in examined women. Statistical analysis of differences in the level of studied cytokines, bone turnover markers and hormones between groups provided various results, ranging from the lack of difference (ns) to significant ($p \leq 0.01$) and in some cases highly significant ($p \leq 0.005$ or $p \leq 0.001$) differences. Significance of individual

Table 5. The level of statistical significance of differences in analyzed cytokines, bone turnover markers and hormones between groups

Parameter	Comparison of groups (significance level p)			
	Group I vs. group III	Group II vs. group IV	Group I vs. group II	Group III vs. group IV
IL-1 β	p = 0.005	p = 0.01	p = 0.001	p = 0.005
IL-6	p = 0.005	p = 0.01	p = 0.001	p = 0.005
TNF- α	p = 0.001	p = 0.001	p = 0.001	p = 0.001
hOST	p = 0.001	p = 0.001	p = 0.005	p = 0.001
CrossLaps	p = 0.001	p = 0.001	p = 0.01	p = 0.005
PTH	p = 0.001	ns	p = 0.001	p = 0.001
D3	p = 0.001	p = 0.005	p = 0.001	p = 0.001
E2	-	-	p = 0.001	p = 0.005
FSH	-	-	p = 0.01	p = 0.01
TSH	ns	ns	p = 0.05	p = 0.05

Table 6. Correlation (r - Pearson constant) between selected cytokines and markers of bone turnover, vitamin D, IBM and BMD

	IL-1 β	IL-6	TNF- α	IBM	BMD
Group I N=35					
hOST	-0.2640	-0.3871	-0.1274	0.4050	0.6952
CrossLaps	0.6070	0.6798	0.5787	-0.7759	-0.7327
D3	-0.4098	-0.7078	-0.3309	-0.3030	-0.1030
Group II N=23					
hOST	0.3260	-0.1756	-0.0500	0.3126	0.5631
CrossLaps	0.2529	0.2244	0.1215	-0.5402	-0.6980
D3	-0.2288	-0.2116	-0.4710	0.4960	0.5460
Group III N=30					
hOST	0.4358	-0.5670	-0.3369	0.4101	0.6743
CrossLaps	0.5989	0.6242	0.7549	-0.6409	-0.7046
D3	-0.2379	-0.3507	-0.1693	-0.4650	-0.4359
Group IV N=25					
hOST	-0.1300	-0.1709	-0.1403	0.8017	0.7846
CrossLaps	0.2511	0.2249	0.2578	-0.5681	-0.6353
D3	-0.4019	-0.4685	-0.2917	0.5003	0.7860

associations is summarized in Table 5.

The differences in the level of cytokines, biochemical markers of bone turnover, vitamin D3 and hormones between groups were analysed using statistical tools. No statistically significant differences in TSH level between group I vs. group III and group II vs. group IV as well as in PTH concentration between group II vs. group IV were found in this study (p=0.05). The results of other analyses differed statistically significant (0.001 < p < 0.05) in all associations presented in Table 5.

Correlations between hOST, CrossLaps and D3 and cytokines and IBM and BMD are shown in Table 6.

In all analysed groups of women, the following associations were observed: a strong positive correlation between IBM and BMD femoral neck (r=0.8001 ÷ 0.8214), a positive correlation between hOST and BMD and IBM, a negative, medium strength relationship between CrossLaps and BMD and IBM; a weak negative correlation between D3 and IBM and BMD. The association between hOST,

CrossLaps and D3 and cytokines was weak. In groups of women with osteoporosis, the increase in CrossLaps was accompanied by an increase in the concentration of cytokines. In groups II and IV, a medium strength, positive correlation between D3 and IBM and BMD was observed. It was not observed a statistical dependence between IBM and age of the examined women.

DISCUSSION

Osteoporosis is a common disease in aging populations (1). It is a chronic disease associated with the deterioration of bone structure microarchitecture. Osteoporosis results in the reduction of bone mass, it slows down bone metabolism, decreases bone strength and predisposes to increased risk of fractures. Numerous studies are carried out in many countries on different continents in order to better understand the pathogenesis of this disease. This problem affects both women and men; however, women are more susceptible to osteoporosis than men. One of reasons for that is a significant decrease in the concentration of oestrogen

during menopause, which leads to the slowing down of bone formation processes accompanied by the increase in bone resorption activity (2, 5, 9). Still, there is no ideal marker of bone turnover. Markers used to assess the ongoing metabolic processes are often influenced by various conditions. Therefore, although it is now possible to assess bone mineral density using a quite reliable method, we still cannot evaluate bone metabolism (10, 11).

Based on our numerous findings, it seems that osteoporosis should be recognized not only on the basis of densitometric parameters. A number of additional tests and proper, comprehensive interpretation of obtained results are also highly important (1). Radioisotopic methods are widely used in the diagnosis of bone system. There are only few published reports concerning the use of these methods in the assessment of bone metabolism in order to evaluate bone quality. The analysis of the accumulation of bisphosphonate tag (MDP) labelled with ^{99m}Tc in skeletal system with the use of appropriate program allows the calculation of IBM which is a derivative of osteoblastic activity (1,6-8,12).

Densitometry is a method widely used in the measurement of bone mineral density. However, it does not evaluate processes that affect bone metabolism. Apart from BMD examination, it is necessary to take into consideration also the results of hormonal, biochemical and imaging analysis which allow for the assessment of osteoporosis development (13-18). Authors are aware that some of results and conclusions contained in this work and concerning bone turnover disturbances in perimenopausal period are not innovative, however, the diversity of research methods and their comparison expands existing routine diagnostics of bone metabolism in pre - and postmenopausal period.

Numerous reports concerning populations of women from various regions of the world have shown that pro-inflammatory cytokines play an important role in bone mass loss (19). In our work, apart from IL-1 β , IL-6 and TNF- α , we analysed also other factors that could influence the process of bone remodelling. The results of these analyses were correlated with a radioisotopic index of bone metabolism - IBM and bone mineral density - BMD.

Higher levels of oestrogen reduce the rate of bone resorption by inhibiting the effect of parathyroid hormone on bone. The concentration of E2 in group I in 100% of patients was below the lower limit of normal and ranged from $0.98 < \text{E2} < 9.14$ mIU/mL.

In 81% of women in group II, the concentration of E2 was in the range of $2.24 < \text{E2} < 9.33$ mIU/mL. In groups III and IV, E2 levels were within the normal range for the analysed age range. The observation that regardless of whether women in groups I and III were menstruating or not, E2 concentration was lower than in groups II and IV was very interesting and required further analysis. Statistically significant differences in E2 levels between group I vs. group II ($p = 0.001$) and group III vs. group IV ($p = 0.005$) were observed in this study. Low concentrations of E2 were accompanied by reduced levels of D3. In group I, D3 values in 93% of women were in the range $9.45 < \text{D3} < 29.19$ ng/mL, in group III in 60% of women they were in the range $21.67 < \text{D3} < 27.22$ ng/mL, in 45% of women in group II - in the range $18.45 < \text{D3} < 27.22$ ng/mL and in 18% of women in group IV - in the range of $24.15 < \text{D3} < 29.76$ ng/mL. Very low levels of E2 in groups of women with osteoporosis were accompanied by increased activity of osteoclasts. The increase in CrossLaps marker and significantly reduced hOST values mirrored this process.

In postmenopausal women, the observed reduction of E2 concentration led to an increase in the activity of pro-inflammatory cytokines: IL-1 β , IL-6 and tumour necrosis factor TNF- α . The observed decrease in BMD and IBM was a result of these changes. In this period, the observed PTH concentration was different between groups and increased but still within the limits of normal. In groups I and III, PTH level was in the range $54.28 < \text{PTH} < 67.87$ pg/mL and in groups II and IV it was lower - $34.15 < \text{PTH} < 41.23$ pg/mL. In group I, PTH level was higher than in group III. The difference in PTH concentration between group I and group III was statistically significant ($p = 0.001$). However, in groups II and IV, there were no statistically significant differences in PTH concentration.

IL-1 β and IL-6 are pro-inflammatory cytokines involved in bone turnover. In our study, the concentration of IL-1 β , IL-6 and TNF- α in all analysed groups, despite differences between groups, were within normal limits. Higher concentrations of cytokines correlated with decreased BMD and was also associated with the reduction of IBM value. However, in groups I and III, levels of these cytokines were higher than in groups II and IV. Many researchers have observed the relationship between the IL-1 β and BMD and bone metabolism. However, the results obtained by them are contradictory and do not lead to clear conclusions (20-23).

The highest concentration of tumour necrosis

factor TNF- α was observed in group I, while the lowest in group IV. At the same time, it was observed that the increase in the concentration of this factor in group I was accompanied by an increase in PTH level and a decrease in the concentration of vitamin D3 at very low concentrations of E2. Normal TSH values in all women ruled out the negative influence of thyroid on bone metabolism and BMD.

The analysis of biochemical parameters indicated that in 14% of women in group I bALP level was below lower limit of normal, ranging from 78.17 <bALP <97.21 U/L. In other groups, bALP values were within the normal range. In 17% of women in group I, P values were also decreased and ranged from 2.36 <P <2.59 nmol/L, while in 19% of women, Ca²⁺ concentration ranged from 0.86 <Ca²⁺ <0.97 mmol/L. Ca²⁺ concentration below normal limit was also observed in 11% of women in Group III.

In all groups, a direct, proportional relationship between hOST and BMD and IBM was observed. In group IV, correlations between hOST and BMD as well as between hOST and IBM were positive and strong. High concentrations of hOST and normal levels of IBM and BMD were the result of high activity of osteoblasts. However, CrossLaps values were inversely proportional to IBM and BMD. All groups, a negative, medium strength correlation was found between CrossLaps and BMD as well as CrossLaps and IBM.

In groups I and III, a weak negative correlation between D3 and IBM and BMD was observed. However, in groups II and IV, the correlation between D3 and IBM and between D3 and BMD was positive and moderate. Despite similar age, bone turnover in postmenopausal women with severe osteoporosis was significantly lower than that observed in menstruating women without osteoporosis. Therefore, it was hypothesized that bone metabolism mainly depended on menopause advancement manifested by changes in cytokines, D3 and PTH (24). In this analysis, the effect of age was less important.

In this study we found a strong, inverse relationship between E2 and PTH levels. The concentration of E2 and D3 was decreasing, while PTH level was increasing with age. Bone metabolism and bone mineral density were also decreasing with age. However, concentrations of analysed cytokines and biochemical markers of bone turnover which mirrored bone metabolism and bone mineral density were closely associated with the decrease in oestrogens and vitamin D3 levels.

In the perimenopausal period, changes in the

level of cytokines, D3 and PTH are observed and at that time they pose the mechanism of bone metabolism disorders and osteoporosis development. The results of the analysis of bone markers hOST, CrossLaps as well as D3 and PTH were various in studied groups and statistically significant differences were observed between group I vs. group II, group I vs. group III and group II vs. group IV.

In perimenopausal women, the increase in pro-inflammatory cytokines may be considered as one of diagnostic indicators of the degree of bone loss and osteoporosis advancement.

Biochemical analysis is only complementary to bone turnover assessment including densitometry and radioisotopic evaluation of bone metabolism. Our study has shown that bone metabolism and osteoporosis depends mainly on menopause advancement and changes in the level of PTH, D3 and cytokines, while age is in this case much less important.

In conclusion, radioisotopic analysis together with densitometry allow for a more accurate assessment of bone metabolism and bone mineral density. Radioisotopic methods provide additional information and offer a chance of early diagnosis of bone metabolism disorders. They are more sensitive in the assessment of bone alterations than BMD.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgement

The study was performed within the investigations and statutory activity of the Department of Diagnostics and Radiological and Isotopic Therapy, Medical University, Lodz.

References

1. Holecki M, Chudek J, Titz-Bober M, Więcek A, Zahorska-Markiewicz B, Duława J. Changes of bone mineral density in obese perimenopausal women during 5-year follow-up. *Pol Arch Med Wewn.* 2012; 122; 4.; 139 – 147
2. Romas E, Gillespie MT, Martin TJ. Involvement of receptor activator of NF κ B ligand and tumor necrosis factor- α in bone destruction in rheumatoid arthritis. *Bone.* 2002;30(2):340–346.
3. Oros S, Ianas O, Vladiu S, Giurcaneanu M, Ionescu L, Neacsu E, Voicu G, Stoicescu M, Rosca R, Neamtu C, Badiu C, Dumitrache C. Does Obesity Protect Postmenopausal Women Against Osteoporosis? *Acta Endocrinologica-Bucharest* 2012; 8(1):67-76.
4. Yilmaz O, Mevsim V, Kuruoglu E. Development of Clinical Risk Assessment Tool for Osteoporosis (Osteoriskapp) Using A Syndromic Approach. *Acta Endocrinologica-Bucharest* 2018; 14(1):133-141.
5. Sunyer T, Lewis J, Collin-Osdoby P, Osdoby P. Estrogen's bone-protective effects may involve differential IL-1 receptor regulation in human osteoclast-like cells. *J Clin Invest.* 1999;103(10):1409-1418.

6. Tryniszewski W, Gadzicki M, Gorska-Chrzastek M, Rysz J, Maziarz Z. Bone metabolism assessment, bone metabolism index designation and the determination of its normal values range in young healthy women. *Med Sci Monit* 2011; 17: CR 563-571.
7. Tryniszewski W, Gadzicki M, Rysz J, Banach M, Maziarz Z. The behaviour of bone mineral density and bone metabolism index in young and menopausal women with the consideration of body mass index. *Med Sci Monit* 2010; 16(7):CR342-347.
8. Tryniszewski W, Sobczuk A, Górka-Chrzastek M, Gadzicki M, Raciborska I, Zając A, Maziarz Z. The assessment of indicator of bone metabolism (IBM) and designation of its normal values range in healthy women and men. *Przegląd Menopauzalny* 2011; 1(53): 50-57
9. Papierska L, Rabijewski M, Kasperlik-Zaluska A, Zgliczyński W. Effect of DHEA supplementation on serum IGF-1, osteocalcin, and bone mineral density in postmenopausal, glucocorticoid-treated women. *Advances in Medical Sciences*, 2012;57;51-57.
10. Korczowska I, Przepiera-Bedzak H, Brzosko M, Lacki JK, Treffer J, Hrycaj P. Bone tissue metabolism in men with ankylosing spondylitis. *Advances in Medical Sciences*, 2011;56(2); 264-269
11. Bogdan Lewandowski, Karol Kita, Jacek Kita, Agnieszka Sulik, Piotr Adrian Klimiuk, Stanisław Sierakowski, Wiesław Zarzycki, Izabela Domysławska. Osteoporoza – część 2. Badanie gęstości mineralnej kości oraz badania obrazowe w diagnostyce osteoporozy, *Borgis - Nowa Medycyna* 3/2004.
12. Thomsen K, Nilas L, Mogensen T, Christiansen C. Determination of bone turnover by urinary excretion of ^{99m}Tc-MDP. *Eur J Nucl Med*. 1986;12(7):342-345.
13. Kanis JA, Borgstrom F, De Laet C, Johansson H, Johnell O, Jonsson B, Oden A, Zethraeus N, Pfeleger B, Khaltayev N.: Assessment of fracture risk. *Osteoporos Int* 2005; 16: 581-589.
14. Raisz LG, Rodan GA. Pathogenesis of osteoporosis. *Endocrinology and Metabolism Clinics of North America*. 2003;32(1):15-24.
15. Chen HY, Chen WC, Wu MC, Tsai FJ, Lin CC. Interleukin-1beta and interleukin-1 receptor antagonist gene polymorphism in postmenopausal women: correlation to bone mineral density and susceptibility to osteoporosis. *Maturitas*. 2003;44(1):49-54.
16. Charatcharoenwittaya N, Khosla S, Atkinson EJ, McCready LK, Riggs BL. Effect of blockade of TNF-alpha and interleukin-1 action on bone resorption in early postmenopausal women. *J Bone Miner Res*. 2007;22(5):724-729.
17. Rahnama M, Jastrzębska-Jamrogiewicz I, Jamrogiewicz R, Trybek G. The Analysis of Hormone Replacement Therapy Influence on IL-6 Expression and Mandible T-Score of Postmenopausal Women. *J Genet Syndr Gene Ther*, <http://dx.doi.org/10.4172/2157-7412.1000253>.
18. Schousboe JT, Bauer DC, Nyman JA, Kane RL, Melton LJ, Ensrud KE. Potential for bone turnover markers to cost-effectively identify and select post-menopausal osteopenic women at high risk of fracture for bisphosphonate therapy. *Osteoporos Int* 2007; 18: 201-210.
19. Czerny B, Kaminski A, Kurzawski M, Kotrych D, Safranow K, Dziedzic V, Bohatyrewicz A, Pawlik A. The association of IL-1beta, IL-2, and IL-6 gene polymorphisms with bone mineral density and osteoporosis in postmenopausal women. *Eur J Obstet Gynecol Reprod Biol*. 2010;149(1):82-85.
20. Tai-Hung Chao, Hsing-Ning Yu, Chi-Chuan Huang, Wen-Shen Liu, Ya-Wen Tsai, Wen-Tung Wu. Association of interleukin-1 beta (-511C/T) polymorphisms with osteoporosis in postmenopausal women. *Ann Saudi Med* 2010; 30: 437-441.
21. Kim JG, Ku SY, Lim KS, Jee BC, Suh CS, Kim SH, Choi YM, Moon SY. Cytokine production by whole blood cells: relationship to interleukin gene polymorphism and bone mass. *J Korean Med Sci*. 2005 Dec;20(6):1017-1022.
22. Kim JG, Lim KS, Ku SY, Kim SH, Choi YM, Moon SY. Relations between interleukin-1, its receptor antagonist gene polymorphism, and bone mineral density in postmenopausal Korean women. *J Bone Miner Metab*. 2006;24(1):53-57.
23. Ozelci R, Dilbaz B, Ozkaya E, Cirik DA, Zengin T, Mollamahmutoğlu L. Association between bone mineral density and metabolic syndrome in Turkish women who were postmenopausal. *Int J Gynaecol Obstet*. 2016 Jun;133(3):370-374.
24. Al-Daghri NM, Yakout S, Al-Shehri E, Al-Fawaz HA, Aljohani N, Al-Saleh Y. Inflammatory and bone turnover markers in relation to PTH and vitamin D status among Saudi postmenopausal women with and without osteoporosis. *Int J Clin Exp Med*. 2014;15(7):3528-3535.