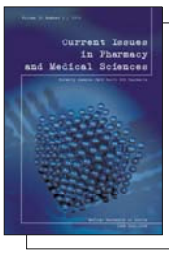

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Osteocalcin and selected biochemical parameters of bone turnover in healthy subjects

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ABSTRACT

Osteocalcin (OC) is non-collagenous bone matrix protein produced by osteoblasts. OC binds to hydroxyapatite and is deposited in the bone matrix. Concentration of OC in the blood seems to reflect both osteogenesis and increased bone metabolism and depends on age, sex, menopausal status. The aim of our study was to evaluate changes in the concentration of osteocalcin and selected biochemical parameters of bone turnover in different stages of life in healthy people. We found that the mean serum levels of OC were significantly greater in women than in men. The highest OC levels were found in men under the age of 30 with a decrease in the fourth decade of life. In the group of women of up to 40 years of age, OC concentrations were comparable with the equivalent group of men. The lowest concentration of OC was found in the age group 40-49, with the significant increase in the subsequent decades of life. In both men and women, we found a significant correlation of OC with the calcium concentrations and alkaline phosphatase activity. Osteocalcin determinationa, particulary in women, may be useful laboratory parameters of osteoporosis process.

Keywords: osteocalcin (OC), bone turnover, age, gender

INTRODUCTION

Despite its rigid structure, bone is a metabolically active tissue, and undergoes continuous remodelling, consisting of two opposing processes: osteogenesis (bone formation) occurs as a result of the action of osteoblasts; and osteolysis (bone resorption) which depends on the activity of osteoclasts. Both processes take place throughout life and are closely related under normal conditions.

The dynamics of these processes of bone formation and resorption change at different times during the normal life span. In the period of rapid growth (adolescence), bone formation is the dominant process, while during the consolidation stage there is a balance between bone formation and resorption. In the involution stage slight increase is usually seen in remodelling in both bone formation and resorption, although there is a tendency for the increase in resorption to be greater (14, 27).

Bone metabolism consists of successive processes of resorption and bone formation and is called bone turnover or bone remodelling and is responsible for the maintenance of constant bone mass, good bone quality and resistance to mechanical forces. The bone remodelling is regulated through the action of numerous factors, for example, mechanical, the action of many systemic hormones and local mediators as cytokines (7, 10, 20, 25).

The purpose of biochemical monitoring of bone metabolism is to assess the balance between the processes of resorption and formation of new bone tissue.

Over the past few years, several new substances were introduced into clinical diagnostics, some of which are the products of osteoblasts metabolism and the others, which are bone degradation products. These substances are known as the "bone markers" and are currently used in routine clinical practice.

For clinical purposes, bone markers are divided into 3 categories:

- bone formation markers, which are substances released by osteoblasts,
- markers of bone resorption, including substances, which indicate osteoclast activity and increased collagen degradation processes,
- markers of bone turnover, released during bone resorption and bone formation.

The aim of this study was to evaluate changes in the concentration of selected bone markers and other biochemical parameters indicating bone turnover in different

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stages of life in healthy people. In particular, our attention was focused on identification of changes in the concentration of a significant marker of bone turnover – osteocalcin (OC).

MATERIAL AND METHODS

The study was conducted in 80 healthy subjects (51 women 29 men) aged 18 -77, attending the periodic health checks at the Department of Laboratory Diagnostics of the Clinical Hospital No. 1 in Lublin. All subjects satisfy the eligibility criterion specified as absence of obvious clinical and laboratory signs or symptoms of any disease, use of medication and drugs or stimulants.

In order to investigate changes in osteocalcin concentrations in subjects, the study group was subdivided by age into 5 categories: 1 group: years, 2 group: 30-39 years, 3 group: 40-49 years, 4 group: 50-59 years, 5 group 60 years.

In all subjects, a single determination of total concentrations of calcium, inorganic phosphate, osteocalcin and alkaline phosphatase was performed in serum. Blood was collected in the fasting state, between 8.00 and 10.00 a.m. in volumes of 7 ml. The obtained serum samples were stored frozen at -20°C until analysis.

For the quantitative determination of osteocalcin in serum enzyme immunoassay ELISA was used (N-MID Osteocalcin ELISA Nordic Bioscience Diagnostics, Denmark). Concentration of total calcium (colorimetric method with o-cresolophtaleine), inorganic phosphate (colorimetric method with ammonium molybdate) and alkaline phosphatase (kinetic method at 37°C, recommended by IFCC) in blood serum were determined using a biochemical analyser Cobas INTEGRA 600 (Roche Diagnostics).

Statistical analysis of the results was carried out using the statistical package STATISTICA 8.0 (StatSoft company).

RESULTS

Table 1 shows the results of determinations of the selected biochemical parameters and OC concentrations in the studied group.

Table 1. Descriptive statistics of selected biochemical parameters and serum OC in the studied group

Parameter	x	SD	Me	Max	Min
Total Calcium (mmol/l)	2.32	0.11	2.31	2.07	2.55
Inorganic Phosphate (mmol/l)	1.07	0.18	1.04	0.64	1.45
Alkaline Phosphatase (IU/l)	66.0	18.1	62.0	31.0	113.0
Osteocalcin (ng/ml)	21.96	9.55	21.86	3.69	42.28

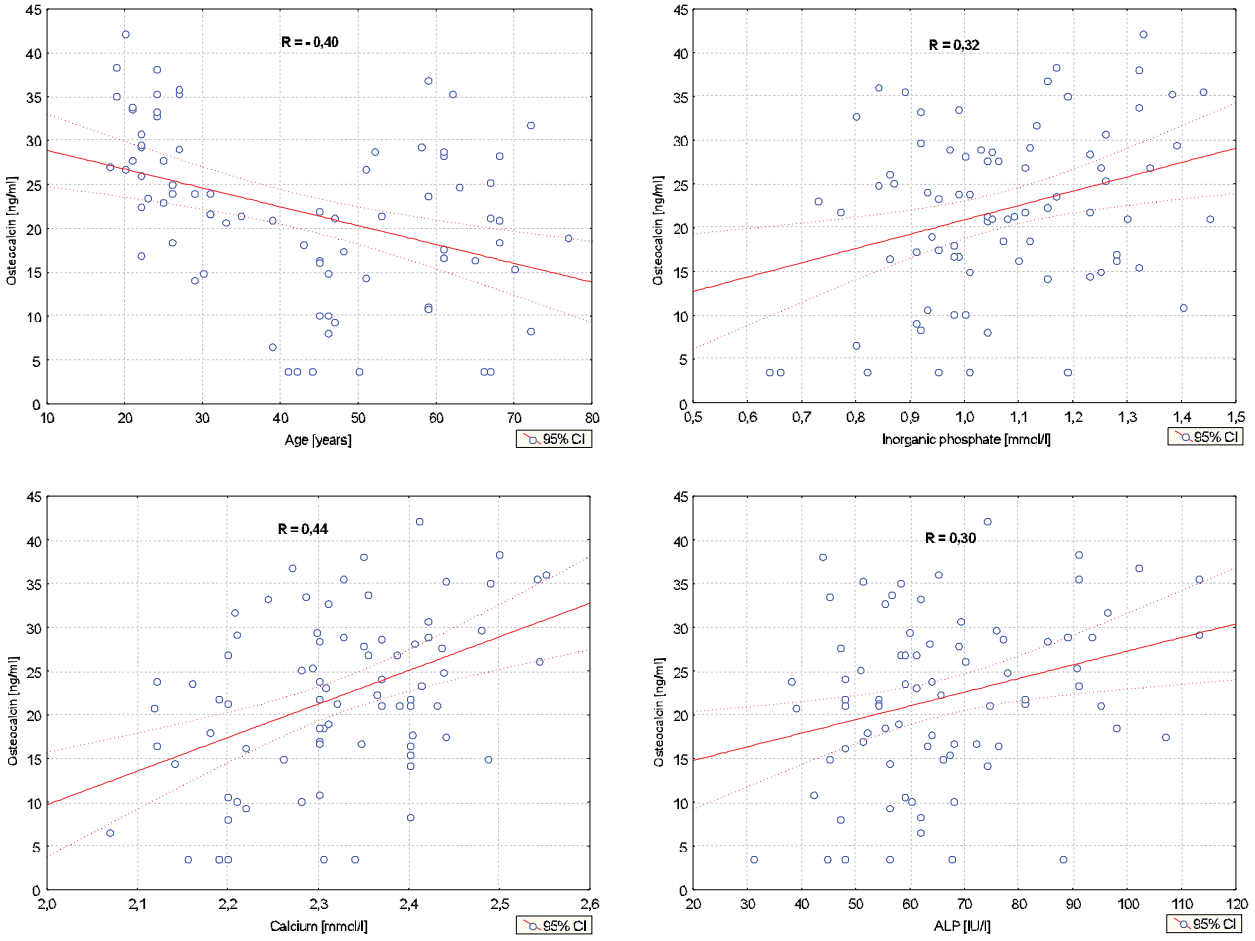

Fig. 1. Correlation of osteocalcin concentrations with subjects' age and values of selected biochemical parameters in the study group

The average OC concentration in the study group was 21.96±9.55 ng/ml and was within the range from 3.69 to 42.28 ng/ml.

The concentration of OC correlated positively with plasma total calcium (R = 0.44, p<0.001), inorganic phosphate (R = 0.32, p<0.01) and alkaline phosphatase activity (R = 0.30, p<0.01). The inverse relationship was found between OC concentration and age (R = -0.40, p<0.001). The relationship between OC levels and the values of selected biochemical parameters and age in subjects are shown in Table 2 and on the Figure 1.

Table 2. Correlation of concentrations of OC with subjects' age and values of selected biochemical parameters in the study group

Parameter	Correlation Coefficient (R)	p
Age	-0.40	0.001
Total Calcium (mmol/l)	0.44	0.001
Inorganic phosphates (mmol/l)	0.32	0.01
Alkaline phosphatase (IU/l)	0.30	0.01

The results of selected biochemical parameters and serum OC determinations in groups of women and men separately are shown in Table 3.

Table 3. The concentration of OC and selected biochemical parameters in the studied groups, broken by gender

Parameters	Women		Men		p
	x	SD	x	SD	
Total Calcium (mmol/l)	2.30	0.10	2.35	0.11	NS
Inorganic phosphates (mmol/l)	1.11	0.17	0.98	0.17	0.01
Alkaline phosphatase (IU/l)	64.0	18.2	69.5	17.7	NS
Osteocalcin (ng/ml)	23.70	8.87	18.91	10.07	0.05

We did not find any gender-related differences in absolute concentrations of total calcium concentration and ALP activity. Values of inorganic phosphate concentrations were significantly higher (p<0.01) in the group of women than in the group of men.

The average OC concentration in women was 23.70 8.87 ng/ml and was significantly higher (p<0.05) when compared with males (18.92±10.07 ng/ml) (Table 3 and Figure 2).

In the group of men we identified a negative correlation of OC concentrations with subject age (R = -0.64, p<0.001). This dependence was not observed in women. In both groups directly proportional relationships of OC with total calcium concentration and alkaline phosphates activity were observed. In the group of men, OC correlated with the inorganic phosphate concentration (Table 4).

In the next stage of our work we investigated subjects (men and women) subdividing them by age into 5 groups: <30 years old, 30-39 years, 40-49 years, 50-59 years, ≥60 years (Table 5, Figure 3). With increasing age in men the

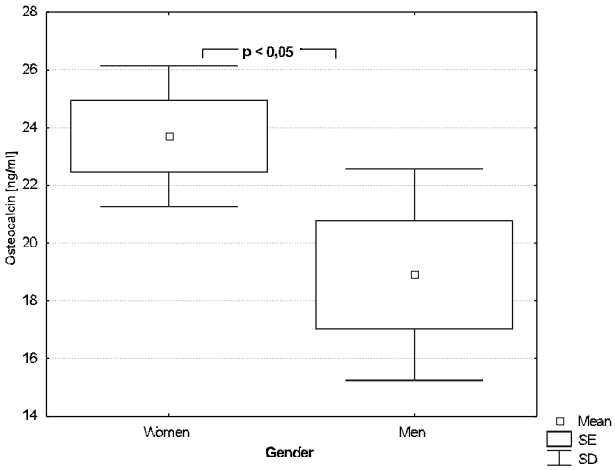

Fig. 2. Osteocalcin concentration in men and women

average OC concentration was undergoing systematic reduction obtaining the value 13.34±7.77 ng/ml in the age group 40-49 years (p<0.001 compared with age below 30) and remained unchanged throughout further stages of life. A similar reduction in serum OC was found in women of up to 50 years of age, whereas in the age group 40-49 years, average osteocalcin concentration was 11.70±5.11 ng/ml and was significantly lower than that of the younger women. In contrast to men, women above 50 years of age showed a significant increase in osteocalcin as compared with younger women, while these levels in women of over 60 were 2-times higher than those found in men in equivalent age group (p<0.001).

Table 4. Correlation of OC concentrations with the selected biochemical parameters and age in the group of men and women separately

Parameters	Women		Men	
	Correlation coefficient (R)	p	Correlation coefficient (R)	p
Age	-0.20	NS	-0.64	0.001
Total calcium (mmol/l)	0.38	0.01	0.70	0.001
Inorganic phosphates (mmol/l)	0.19	NS	0.37	0.05
Alkaline phosphatase (IU/l)	0.35	0.05	0.37	0.05

Table 5. Osteocalcin concentrations in studied men and women subdivided by age group.

Age group	Women			Men			P value
	N	x	SD	N	x	SD	
< 30	20	28.63	7.54	9	29.76	4.74	NS
30-39	7	18.36 b	5.70	1	23.91	0.00	NS
40-49	7	11.70 a,c	5.11	7	13.34 a	7.77	NS
50-59	7	23.41 d	8.80	3	14.42 b	12.94	NS
≥ 60	10	26.19 c,e	5.63	9	13.32 a	6.29	0.001

a – p<0.001– compared with age years
b – p<0.01 – compared with age years
c – p<0.05 – compared with the age group 30-39 years
d – p<0.05 – compared with the age group 40-49 years
e – p<0.001– compared with the age group 40-49 years

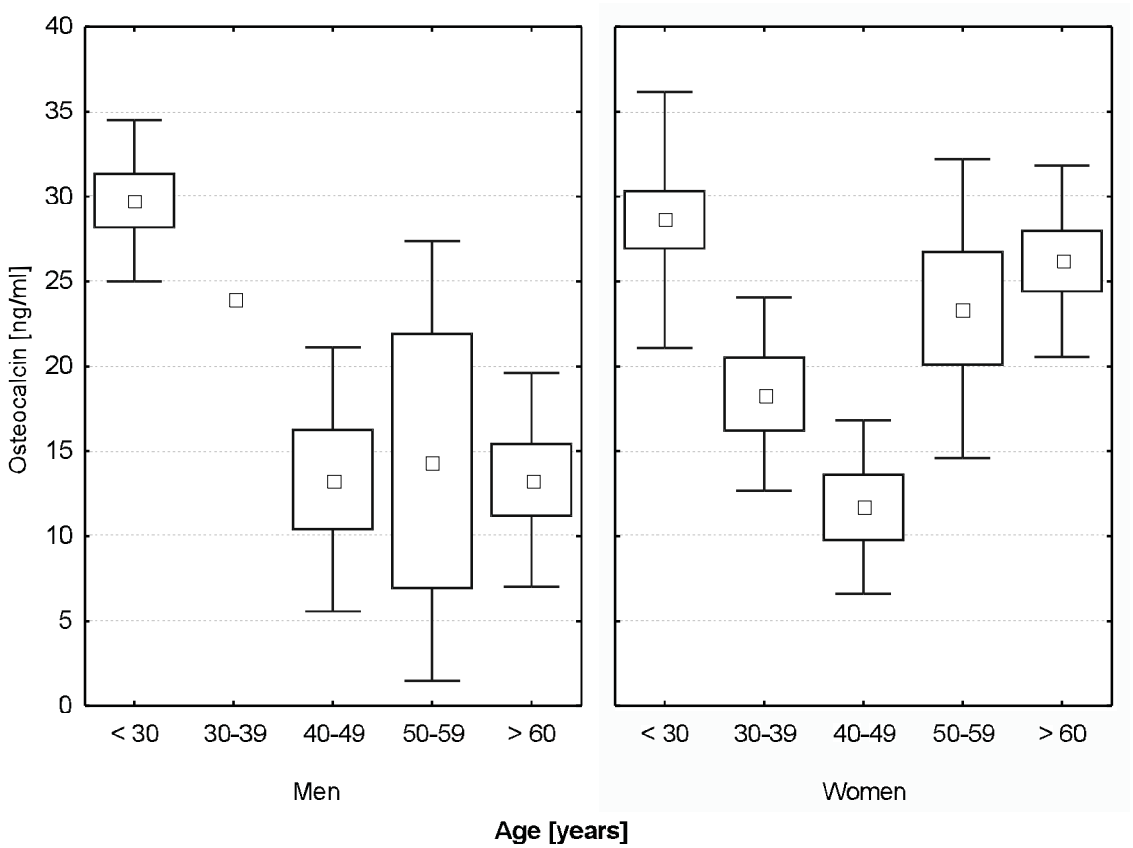

Fig. 3. OC concentrations in studied groups of men and women broken by age

DISCUSSION

Biochemical markers of bone turnover are fragments of proteinaceous structural elements of bone (or their degradation products), as well as enzymes and proteins released into the circulation during metabolic activity of bone forming cells (osteoblasts) and during osteoclastic bone resorption. Their concentration measured in serum and urine is the result of activity of all remodelling processes taking place at a given time throughout the whole skeleton. Determination of concentration of the biochemical markers of bone turnover allows the assessment of bone formation and resorption process rates. This enables the prediction of risk of fragility fractures and bone mass changes in the course of treatment (5, 12, 14, 18, 19, 20, 26).

In this study, among the different biochemical parameters reflecting bone metabolism, particular attention was paid to the osteocalcin (OC) – non-collagenous bone matrix protein produced by osteoblasts.

OC is released into the systemic circulation in bone formation as well as in bone resorption processes. Hence, it is considered one of the new indicators of metabolic activity of osteoblasts and the bone resorption process (2, 9, 11, 12, 13, 21, 24, 25).

The physiological function of OC, at this time, is not exactly known. In vitro and in vivo studies conducted by Chenu C. et al demonstrated OC role in the osteoclasts chemotaxis, their migration and adhesion to the surface of mineralising bone (4) and in the regulation of bone turnover and activation of osteoblasts (2). Research of Vergnaud et al. (28) suggests that the function of OC is the initiation of bone resorption and maintaining of trabecular bone remodelling.

Jilka et al. (17) showed that concentrations of OC in serum depend on age, sex, menopause status and the course of the skeletal maturation.

In children, bone turnover may be 10 times greater than in adults because there are three very active processes at work: bone modelling, remodelling and growth.

It has been reported that the concentration of OC in children is high and correlates with the rate of growth. Whereas in perimenopausal women and in men over 60, the concentration of OC in the serum is elevated (23, 25).

Results of our studies revealed the highest concentration of OC in both women and men under 30 years of age. In men over 40 years of age, the concentration of OC substantially declined and remained constant in subsequent years of life. The lowest concentrations of OC were found in women between 40-49 years of age. In subsequent decades of life, concentrations of OC increased significantly.

Jancewicz P. et al. (16) who conducted the research in a group of 179 healthy women obtained similar results. Moreover, these authors showed reduction in serum OC in women between 30 and 49 years of age and a significant increase after the age of 50.

This characteristic increase in the OC concentration in 50-year-old women is linked to hormonal changes occurring in the female body. The decrease of sex steroids secretion, mainly of oestradiol in postmenopausal women, results in increased osteoclast activity and thus increased bone resorption. Lack of these hormones in various forms of hypogonadism, results in osteoporosis. The oestrogens have important impact on bone metabolism in women. This effect on bone is to mediate changes in concentrations of various mediators acting systemically and locally, such as calcitonin (for which receptors are located on osteoclasts), interleukin 1 and 6, IGF (Insulin-like growth factor), TNF. It makes estrogens protective from bone resorption through inhibition of macrophage development and reduction of the proliferation of osteoclast precursors. Oestrogens also increase osteoclast apoptosis, stimulate proliferation and activity of osteoblasts. The presence of osteoporosis in people over 60 years of age therefore might be explained by hormonal changes (18, 19 ,20).

The imbalance in the metabolic processes occurring in bone is also associated with abnormalities in plasma calcium levels (27).

In our study group, we found a positive significant correlation between serum OC concentrations and the levels of calcium and inorganic phosphate. Lepage O.M. et al., (22) in studies performed in horses, have shown the statistically significant relationships between the concentrations of osteocalcin and inorganic phosphate levels, however they did not observe a correlation between the OC and calcium concentrations.

Despite the lack of tissue specificity, the determination of alkaline phosphatase (ALP) activity in serum is the most often used marker of bone formation. ALP is an enzyme present in many tissues, but most of its activity is found to be in bone cells, liver, small intestine, placenta and leukocytes (9, 12, 15, 18, 19).

Bone izoenzyme of ALP is synthesized by osteoblasts and released into the bloodstream in the middle stage of the bone formation process, i.e. during maturation of bone matrix. It is believed that this enzyme plays an important role in the initiation of bone mineralization and affects the early stages of its progress. Bone izoenzyme of ALP is considered a marker of bone formation with a high degree of specificity, and its additional valuable feature is the long enzyme-molecule half-life in the blood stream (1-2 days), leading to a small daily variation in this parameter (1, 3, 8, 14, 15).

Bone development causes a multiple (3-4 times in infants and 2-3 fold in children and adolescents) increase in the total activity of alkaline phosphatase in serum in comparison with the reference ranges for adults (18, 19). In the serum of the elderly, a 2-fold increase of ALP was found. Activity of this enzyme increases in all diseases characterized by increased osteoblastic activity, as well as in cancer. In these cases, measurements of total ALP activity are reliable. However, in osteoporosis, where the osteoblast activity is only slightly increased, the contribution of the extracellular fraction of ALP may be sufficiently significant, such that the reliability of the results may be doubtful.

In the present study, we found the significant positive correlation between ALP activity and OC levels in both women and men. A similarly significant positive correlation between ALP activity and OC was described by Lepage O.M. et al. (22). Moreover Lian J.B. et al. (23) noticed that changes OC concentration throughout a man's life strikingly resemble ALP activity, with higher values during development, aging and lower during the equilibrium states.

Taking together, results of our research indicate that the OC determinations are a valuable source of information about bone formation processes occurring in the organism. However, the correct interpretation of the results requires knowledge of the OC level variability over a man's life span.

CONCLUSIONS

1. In women, the mean serum OC was significantly greater than those reported in men.

2. The highest OC levels are found in men under the age of 30 with a decrease in the fourth decade of life, when the concentration remains stable for the subsequent years.

3. In women of up to 40 years of age, OC concentrations are comparable with a equivalent group of men. The lowest concentration of OC is found in the age group 40-49 years, with the significant increase in the subsequent decades of life, what may be linked with estrogens status and increased risk of osteoporosis.

4. In both men and women, we found a significant correlation of OC with the calcium concentrations and alkaline phosphatase activity.

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