Research Article

New phenomena in the clinical and morphological aspects of osteoporosis

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ABSTRACT

Objective: The aim of this study is to compare the biochemical indexes of phosphorus-calcium (Ca) metabolism in patients with metabolic syndrome of 1–5 stages, associated with osteoporosis with morphological picture of bone tissue. **Materials and Methods:** A total of 248 patients (99 men and 149 women) in the age from 60 to 69 (mean age was 64 ± 3.5) were examined during the study. **Results:** The results of the study of biochemical indexes of phosphorus-calcium (Ca) metabolism in patients with metabolic syndrome associated with osteoporosis were presented in the article. The clinical picture is compared with electronic microscopically features of bone tissue and indexes of elements in it. It was shown that phosphorus-Ca imbalance in patients with metabolic syndrome has already determined on the 2nd stage of the metabolic syndrome and it is represented by decreasing of 1.25 (OH)2 Vitamin D and increasing of parathyroid hormone in blood serum, what is associated with beginning of osteoporosis. **Conclusion:** Bone mineral density is decreased in patients with metabolic syndrome of 1–4 stages as metabolic syndrome progresses. Osteoporosis/osteopenia is more expressed in bones of the forearm, and it is connected with hyperparathyroidism in patients with metabolic syndrome of the 5th stage.

KEY WORDS: Bone mineral density, Osteoporosis, Phosphorus-calcium metabolism

INTRODUCTION

Osteoporosis is one of the important problems of health care, as its frequency constantly grows.[1] The social significance of osteoporosis is determined by the high prevalence of this pathology and mortality from its outcomes - fractures of femur and spine, loss of ability of work and self-service, bigger economic costs on prophylactics, treatment, and service. In city population, 24% of women and 13% of men at the age of 50 and over have already had fractures according to the data of federal center of prophylactics of osteoporosis.[2,3] Nowadays, more than 200 million people are suffered from osteoporosis. According to estimation in the Russian Federation, 14 million people (10% of country's population) are suffered from osteoporosis, and another 20 million people have osteopenia. Thus, 24% of citizens of Russian Federation (34 million people) enter into a group of the potential risk of osteoporotic fractures.[4] It is known that one osteoporotic fracture takes place in every third woman in the age over 50.[4,5] Annually, the frequency of femur fractures is 105.9 per 100,000 population, and the frequency of fractures of the distal part of the forearm is 462.2/100,000 population.[5] According to statistic data, 10% loss of bone mineral density (BMD) in bodies of vertebra leads to increasing of the risk of their fractures in 2 times, and the similar decreasing of BMD in femur leads to increasing of distal femur fracture in 2.5 times.[6]

The Aim of Work

The aim of this study is to compare the biochemical indexes of phosphorus-calcium (Ca) metabolism in patients with metabolic syndrome of 1–5 stages, associated with osteoporosis with morphological picture of bone tissue.

MATERIALS AND METHODS

A total of 248 patients (99 men and 149 women) in the age from 60 to 69 (mean age was 64 ± 3.5) were examined during the study. Metabolic syndrome divided into stages depending on the degree of obesity: 1 stage - metabolic syndrome with pre-obesity (increased normal body weight); 2 stage - metabolic syndrome with the obesity of 1° (51 patients); 3 stage - metabolic syndrome with the obesity of 2°

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(46 patients), 4 stage - metabolic syndrome with the obesity of 3° (38 patients), and 5 stage - metabolic syndrome with the obesity of 4° (56 patients).

Next, indexes were estimated in patients: Height, weight, arterial pressure, cholesterol and glucose statuses, presence of heart insufficiency and its expression according to NYHA classification, common laboratory indexes, serum electrolytes, including ionized and albumin-corrected Ca (which can be counted by formula Ca corr.=Ca total+([40–albumin in g/l] × 0.02]), product of Ca×phosphate (P), total alkaline phosphatase (AF), lipidogram, C-reactive protein, fibrinogen, and parathyroid hormone (PTH). Daily proteinuria, daily excretion, and excreted fractions of urea, creatinine, and electrolytes were determined. 25(OH)D и $1.25(OH)_2D$ of blood serum, osteocalcin, C-telopeptide of collagen type 1, and genetic polymorphism of Vitamin D receptor were researched by with help of a set of company "IDS."

Dual-energy X-ray absorptiometry of three parts of the skeleton (lumbar vertebrae L1-L4, proximal part of femur, and distal part of forearm) for the determination of BMD was performed on device Hologic QDR 4500C. BMD was estimated by T-criteria (number of RMS deviations from peak bone mass of healthy people) and Z-criteria (RMS deviation from bone mass of healthy people of same age, sex, and race).

The degree of decreasing of bone mass on radiographs of the wrists was determined by the thickness of two cortical layers of second metacarpal bone. It was calculated by formula (bone diameter in the middle of diaphysis minus diameter of medullary canal) and by the Barnett–Noordin index (the ratio of diaphysis diameter to thickness of cortical layers).[7]

The specimens, taken after surgery for light microscopy, were poured into paraffin blocks. The specimens were viewed and photographed in light microscope TOPIC-T ("CETI Medline Scientific Ltd," Great Britain). The samples were viewed in raster microscope "FE Quanta 200 3D" and "FE1 Quanta 600 FEG" (Netherlands) with the function of non-contact determination of the percentage of macro- and microelements. Macro- and microelemental analysis was performed with use of a detector for the registration of specters of characteristic X-ray radiation (company "EPAX"). Detectors were integrated with raster electron microscope "Quanta 600 FEG." The study was based on the appearance of continuous fluorescent radiation at bombarding of researched samples by a beam of primary X-ray.

The estimation of microvolumes of substance is the method of X-ray spectral analysis. Microfocus X-ray tube, combined with optical microscope, is the base of microanalyzer. Special electron optical system makes thin electron probe, which bombarding small area (1–2 μm) of examined section located on the anode. Microspectral analysis is conducted by spectrograph with curved crystal for the determination of several elements in defined point of section and for exploration of the distribution of one of them across chosen direction. The exploration of the defined surface of analyzed specimen by electron probe displays on the screen the distribution of chemical elements on surface of section. Absolute sensitivity of the method is 10–13–10–15 g. The fault at elemental analysis is 0.2–0.25% (by concentration). The exploration of the content of macroelements (Ca, oxygen, sodium, potassium, magnesium, phosphorus, sulfur, nitrogen, and chlorine) and microelements (fluorine and silicon) was conducted.[8,9]

The statistic processing of material was performed with use of standard programs of applied statistical analysis (Statistica 6.0). Methods of descriptive statistics (mean arithmetic, median, and mean square deviation), Student's *t*-test, and the Wilcoxon Mann–Whitney U-test, the Pearson's χ^2 criteria, single-factor variance analysis (ANOVA), linear correlation analysis (Pearson's r-criteria and Spearman's Rs-criteria), and multifactorial methods (multiple linear regression analysis and discriminant analysis) were used. The numeric data are given as mean values with a standard deviation (M±SD), mean values are shown on the bar graphs, and the vertical lines reflect a 95% confidence interval. Critical level of reliability of the null statistical hypothesis was 0.05.[10]

RESULTS AND DISCUSSION

Beginning from 2nd stage of metabolic syndrome, the level of PTH in serum grew as metabolic syndrome progressed (in comparison with 1st stage $P = 0.013$). Such early increasing of the level of PTH was detected for the 1st time. The tendency to decrease the physiologically active ionized Ca was detected as metabolic syndrome progressed, and besides its level on 5th stage was lower than on 4th stage ($P = 0.032$). The significant decreasing of daily excretion of Ca was observed at increasing of chronic heart insufficiency ($F=17.8$; $p_{ANOVA} < 0.001$).

The excreted fraction of Ca had a tendency to increase on 4th stage in comparison with 3rd stage ($P = 0.051$), and it increased rapidly on 5th stage (in comparison with 4th stage). The phosphorus of serum decreased on 2nd stage in comparison with 1st stage ($P < 0.001$), it began to increase from 4th stage. The daily excretion of P with urine decreased from 4th stage of metabolic syndrome ($F=9.6$; $p_{ANOVA}<0.001$). The excreted fraction of P had increased quickly since 2 stage of metabolic syndrome (in comparison with 1 stage $P = 0.001$). The decrease of the level of $1.25(OH)_2D$

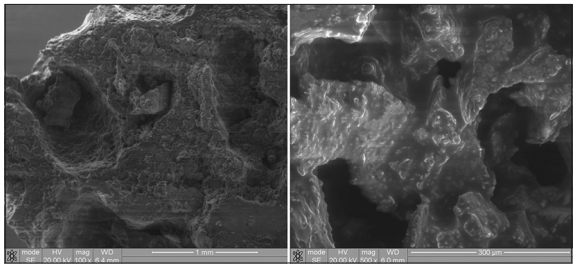

Figure 1: (a and b) Fragments of bone tissue at osteoporosis

in serum was observed due to decrease of its synthesis in renal tubules in consequence of nephrosclerosis and hyperphosphatemia (Rs = −0.26; $P = 0.016$).

The values of 25(OH)D were stable on all stages of metabolic syndrome (F=0.3; $p_{ANOVA} = 0.9$). According to the results of multiple regression analysis, the level of PTH was higher in patients who had high functional class of chronic heart insufficiency ($t = 6.70$; $P < 0.001$), low level of Ca ($t = 1.97$; $P = 0.052$), and more higher level of P in serum ($t = 2.41$; $P < 0.016$). Daily excretion of Ca ($t = 4.09$; $P < 0.001$) and P ($t = 1.78$; $P = 0.076$) was higher in patients with higher level of $1.25(OH)_2D$ in blood ($R^2 = 0.24$; F = 12.9; $P < 0.001$). This dependence can be explained by the fact that $1.25(OH)_2D$ stimulates absorption of Ca and P in intestine and resorption of Ca from bones, what finally determines the amount of excretion of these substances by the kidneys. The concentration of $1.25(OH)_2D$ directly depended ($R^2 = 0.19$; F = 10.0; $P < 0.001$) from functional class of chronic cardiac insufficiency ($t = 3.42$; $P = 0.001$) and values of 25(OH)D in serum ($t = 1.89$; $P = 0.068$). Thus, the insufficiency of 25(OH)D as a substrate of active form of Vitamin D - $1.25(OH)_2D$ - negatively influences on level of last one in some degree. Feedback of level $1.25(OH)_2D$ with daily proteinuria (Rs = −0.27; $P = 0.012$) can be considered as antiproteinuric, nephroprotective effect of D-hormone. The value of Ca in blood was determined ($R^2 = 0.09$; F = 5.35; $P = 0.025$) only by the level of $1.25(OH)_2D$ in serum ($t = 2.31$; $P = 0.024$). Daily excretion of P ($R^2 = 0.08$; F = 4.4; $P = 0.009$) also depended only from the level of D-hormone in blood ($t = 2.76$; $P = 0.024$). As chronic cardiac insufficiency progressed, excreted fractions of Ca and P were increased (Rs = −0.22; $P < 0.001$ и Rs = −0.71; $P < 0.001$), and besides the last one directly correlated with level of PTH in serum (Rs = 0.51; $P < 0.001$). Consequently, the decreasing of daily excretion of Ca and P, probably, connected with decreasing of production of $1.25(OH)_2D$ during progression of metabolic syndrome. Significant growth of excreted fraction P is conditioned by increasing of PTH in serum.

In patients, examined in period April–June, the level of 25(OH)D was significantly lower than in September–December (55.7 ± 46.4 and 91.6 ± 47.7 pmol/L, $P = 0.002$). There were no season differences of $1.25(OH)_2D$. The deficit of 25(OH)D (<37.5 mmol/l) was detected in 25.9%, the deficit of $1.25(OH)_2D$ (<53.2 pmol/L) was in 61.5% of patients, and the insufficiency of 25(OH)D (below the optimal level - 75 pmol/l) was in 77.9% of patients.

Mean value of BMD of three parts of the skeleton was determined by dual-energy X-ray absorptiometry in 192 patients with the metabolic syndrome of 1–4 stages and in 56 patients with metabolic syndrome of the 5th stage. According to T-criteria, the share of patients with normal BMD was 61.3%, patients with osteopenia - 26.3%, and patients with osteoporosis - 12.7%. In group of patients with chronic heart insufficiency of 4 functional class – 35.9%, 31.3% and 32.8% respectively. In patients with metabolic syndrome of 5th stage, BMD of the forearm and proximal part of the femur were significantly lower in comparison with another group ($P < 0.001$), and the tendency to more lower values of BMD of lumbar vertebrae was also observed ($P = 0.08$). The values of BMD of the forearm were the lowest and BMD of vertebrae were maximal (in comparison with forearm $t = 1.98$; $P = 0.047$) in this group. In group of patients with metabolic syndrome of 1–4 stages, there was a tendency to lower values of BMD of the forearm in comparison with proximal part of femur ($P = 0.08$).

BMI directly influenced on BMD of vertebrae in patients with metabolic syndrome of 5th stage (Rs = 0.41; $P < 0.001$). The connection with gene polymorphism of the receptor of Vitamin D TAQI was revealed: BMD was higher in patients with genotype IT in comparison with genotypes Tt and TT. Gene polymorphisms such as BSMI and APAI did not influence on BMD. According to the data of correlative analysis, BMD of the proximal part of femur was lower in women (in comparison with men) (Rs = −0.26; $P = 0.047$), in patient with lower BMI (Rs = 0.54; $P < 0.001$), in patients with ischemia of myocardium on electrocardiography monitoring (Rs = −0.24; $P = 0.047$), and in cases of expressed heart insufficiency (Rs = −0.32; $P = 0.015$). It is necessary to note that BMD of the proximal part of the femur was lower in patients with mitral stenosis in comparison with patients without this pathology ($t = 3.18$; $P = 0.004$).

Values of BMD of forearm correlated directly with body mass of patient (Rs = 0.42; $P < 0.001$). The feedback was revealed with degree of expression of chronic cardiac insufficiency (Rs = −0.46; $P < 0.001$) and with indexes, which reflected speed of metabolism in bones and expression of hyperparathyroidism (levels of PTH (Rs = −0.36, $P = 0.003$), AF (Rs = −0.55, $P < 0.001$), osteocalcin (Rs = −0.39; $P = 0.018$), and C-telopeptides of type I

collagen (Rs = −0.53, $P < 0.001$). Thus, at the chronic cardiac insufficiency of 4th functional class (as a result of metabolic syndrome), BMD of forearm decreased more significantly in comparison with the proximal part of femur, while densitometric parameters of vertebrae did not change. Perhaps, the loss of bone mass in the periphery of the skeleton was conditioned by insufficient correction of hyperparathyroidism.

The direct link of BMD of forearm with BMI (Rs = 0.43; $P < 0.001$), functional class of chronic cardiac insufficiency (Rs = 0.22; $P = 0.018$), and level of hemoglobin (Rs = 0.21; $P = 0.027$) were detected in patients with metabolic syndrome of 1–4 stages at correlative analysis. The feedback of BMD of the forearm with the product of Ca×P of blood on the level of tendency was revealed (Rs = −0.18; $P = 0.08$). The revealed decreasing of BMD at the progression of chronic cardiac insufficiency ($F = 6.2$; $p_{ANOVA} = 0.003$) can be explained by the progression of secondary hyperparathyroidism and decreasing of $1.25(OH)_2D$ concentration.

In patients with metabolic syndrome, the bone density decreases as metabolic syndrome progresses. Osteopenic alterations are more expressed in bones of the forearm and associated with the development of hyperparathyroidism. The content of potassium and phosphorus increased on 1st and 8th weeks in matrix bone (6.58 ± 1.34%; 7.05 ± 1.84%) (2.38 ± 0.04%; 3.53 ± 0.45%) and newly formed bone (14.93 ± 2.94%; 10.04 ± 2.74%; 3.77 ± 0.01%; 5.71 ± 0.30%); the content of potassium and phosphorus in comparison with undamaged bone (5.46 ± 1.02%; 2.01 ± 0.02%).

The structure of bone tissue is violated. Cavities with sides of necrosis and fragments of destructed tissue were observed [Figure 1a and b].

The content of hydrophobic elements in mineral component of humerus, Ca and P, increased from 13.54 ± 0.37% to 21.07 ± 0.53% and from 14.35 ± 0.23% to 21.13 ± 0.15%, respectively. Herewith, the ratio Ca/phosphorus increased from 0.94 ± 0.03% to 1.06 ± 0.04% in period from 7 to 90 days.

Thus, phosphorus-Ca imbalance in patients with metabolic syndrome was determined on the 2nd stage when chronic cardiac insufficiency of 1 functional class took place as a result of metabolic syndrome. It is manifested by decreasing $1.25(OH)_2$-Vitamin D and increasing of PTH in blood serum, what is associated with the beginning of osteoporosis. The level of phosphate decreased transiently due to increasing of PTH in blood, after that it progressively grew, what allowed to note leading role of violation of phosphate metabolism in the development of hyperparathyroidism. In patients with metabolic syndrome of 1–4 stages, BMD decreases as metabolic syndrome progresses. Osteopenia/osteoporosis was more expressed in bones of the forearm and connected with hyperparathyroidism in patients with metabolic syndrome of the 5th stage. The data of biochemical studies are comparable with information, taken with the help of electron microscopy.

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