

TARTRATE-RESISTANT ACID PHOSPHATASE AND OSTEOCALCIN IN PATIENTS WITH OSTEOPOROSIS AND OSTEOPENIA

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Summary: A way to estimate the activity of the osteoclasts is to measure the catalytic activity of the tartrate-resistant acid phosphatase (TRAP), as the bone cells secrete it during the resorption. Osteocalcin (OC) is a non-collagen peptide involved in the bone mineralization process. It is used in early diagnosis of the primary osteoporosis. The aim of our work was to determine if there was a difference in both the catalytic activity of the TRAP and the serum concentration of OC in patients affected by osteoporosis and osteopenia. The bone mineral density T-score (BMD) of the first group of patients (n=60) was not higher than -2.3, defined by the WHO as osteoporosis. The T-score of the second group (n=60) was found to be between -0.4 and -1.7, defined by the WHO as osteopenia. The catalytic activity of TRAP was determined by the kinetic method proposed by the BIOMERIEUX Company (Germany), using a Mira Cobas plus analyzer (Germany). The BMD was determined by an ultrasound technique. The ROCHE ECL technique and the Elecsys 2010 analyzer (Germany) were used to measure the OC concentration. The results show a significant increase in the catalytic activity of TRAP in the osteoporosis patients sera when compared with the group with osteopenia ($p < 0.001$). Statistically significant difference was found between the groups in the case of OC ($p < 0.01$). We conclude that the TRAP catalytic activity in serum, as well as OC concentration measurement, together with other biochemical markers of bone turnover, can be useful in the diagnosis of osteoporosis.

Key words: tartrate-resistant acid phosphatase, osteocalcin, osteoporosis, osteopenia

Introduction

Bone turnover is essential for bone health. It is a process characterized by two tightly coupled activities: resorption of old bone by osteoclasts followed by the formation of new bone by osteoblasts. Bone formation is much slower than resorption and takes place over approximately 90 days in healthy individuals (1). After middle age, in some cases even earlier, bone loss occurs because resorption exceeds formation. The rate of resorption and formation of bone matrix can be assessed by measuring the bone matrix components released into circulation during remodeling, i.e. the biochemical markers of bone turnover (2, 3). An ideal marker, if there were such, would be specific for the bone tissue and stable over at least several days. It would not be subject to assay imprecision

or interference. It would take into account variability (preanalytic, analytic, intra- and interindividual) (4).

Osteoporosis is the most frequent metabolic bone disease (5). According to the definition of the World Health Organization (WHO), osteoporosis is characterized by decreased mineral density, changes of microarchitecture and reduced biomechanical properties of bones that could lead to bone fractures and deformities (6).

Metabolic markers of bone metabolism may be useful for the diagnosis and monitoring of bone diseases, such as osteoporosis (7). Nevertheless, opinions of authors involved in this topic differ very much, from those believing that biochemical markers are insufficiently specific parameters for the diagnosis of metabolic bone diseases, to those claiming that recently detected markers of bone turnover can significantly improve the diagnostic potential of these diseases (8–12). These controversies and dilemmas prompted us to study tartrate-resistant acid phosphatase (TRAP) and osteocalcin (OC) as biochemical markers of bone turnover in patients with osteoporosis and osteopenia.

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Acid phosphatase is a lysosomal enzyme found in bone, prostate, platelets, erythrocytes and spleen. Of the five isoenzymes of acid phosphatase, the bone isoform is tartrate-resistant (TRAP) but unstable. TRAP can be measured in serum or plasma by electrophoresis (after treatment with tartrate) or by immunoassay. Serum acid phosphatase concentrations are typically higher than those in plasma because of the release of acid phosphatase from erythrocytes during clotting (2). Determination of catalytic TRAP activity in blood serum estimates osteoclastic activity, because it is secreted by cells during bone resorption (13).

Osteocalcin (OC) is a small 49-amino acid protein, rich in glutamic acid. It is the major non-collagen protein of bone matrix. In addition to bone, it is also found in dentin. OC is considered to be a sensitive and specific marker of bone synthesis, and it has been successfully applied in monitoring antiresorptive therapy of osteoporosis (14).

Material and Methods

Patients. This study included 120 patients divided into two groups. The first group (n=60) consisted of patients (34 to 77 years old, average age 54.2 years; 50 women and 10 men) with diagnosed osteoporosis confirmed in the out-patient department of the Clinic for Orthopedics and Traumatology, Clinical Center, Banja Luka. According to the WHO standards, bone mineral density (BMD) served as a criterion for the diagnosis. The BMD of ≤ -2.3 in this group determined by an ultrasonic technique was compared with that of young and healthy population (control) using the T-score (standard deviation). According to the WHO standards, T-values from -2.5 to -1 point to osteopenia, as found in the second group of patients (n=60; T-values ranging from -1.7 to -0.4) consisting of 49 women and 11 men, average age 46.1 years, age range from 18 to 66 years.

Determination of serum TRAP activity. Catalytic TRAP activity in the blood sera was determined by the kinetic method, using BIOMERIEUX reagents (Germany) and a Mira Cobas plus analyzer (Germany). α -Naphthyl phosphate dissolved in citrate buffered solution served as a substrate. After the incubation period, the amount of evolved α -naphthol in the presence of a diazonium salt Fast Red TR was measured colorimetrically, with the reference range from 1.7 to 3.2 U/L.

Measurement of OC levels. Concentrations of serum OC were determined by the electroluminescent technique (ECL) using reagents produced by ROCHE (Germany) and an Elecsys 2010 analyzer (Germany), with the reference concentration range from 11 to 43 ng/mL.

Results

As described in *Table I*, catalytic TRAP activities were 5.49 ± 1.63 U/L and 3.51 ± 1.14 U/L in the blood sera of patients with osteoporosis and those with osteopenia, respectively. The difference in the activity of this enzyme between these two groups was of high statistical significance ($p < 0.001$). Comparison of these results with the reference range of 1.7 to 3.2 U/L revealed statistically significant difference only between the values found in examinees with osteoporosis ($p < 0.001$), while that observed between TRAP activity in patients with osteopenia and the reference values was statistically insignificant.

Serum OC level was higher in the group of patients with osteoporosis in relation to the group with osteopenia (32.07 ± 6.24 ng/mL vs. 29.26 ± 3.65 ng/mL) and the difference was statistically significant ($p < 0.01$). However, no statistically significant differences were observed between either of the two groups and the corresponding reference values.

Table I Catalytic activity of tartrate-resistant acid phosphatase and levels of osteocalcin in the blood sera of patients with osteoporosis and osteopenia.

	Tartrate-resistant acid phosphatase (U/L)	Osteocalcin (ng/mL)
OSTEOPOROSIS (n=60)	5.49 ± 1.63	32.07 ± 6.24
OSTEOPENIA (n=60)	3.51 ± 1.14	29.26 ± 3.65
	$p < 0.001$	$p < 0.01$

Discussion

The estimate of a possible risk for bone fractures correlates with the loss of bone mass, but it is difficult to distinguish between osteopenia and osteoporosis, taking into account the upper limit of the T-score of -2.5 . Numerous prevention measures related to osteoporosis development became quite known to the public (15), but it seems that osteopenia remained somewhat neglected with this regard. It is of great importance, therefore, to improve the diagnostics of osteoporosis and osteopenia using reliable biochemical parameters (1).

With respect to bone diseases, biochemical markers represent the molecules directly connected to both the structure and function of bone tissue. The fact that the changes in either the concentration or activity of these biochemical markers are reflecting the dynamic status of bone metabolism is taken as advantageous. There are three groups of biochemical markers of bone turnover. The first group that includes the markers related to calcium and phosphorus homeostasis has been used for a long time in laboratory diagnostics. The second group of biochemical markers consists of enzymes reflecting the

activity of osteoblasts and osteoclasts, while recently identified markers of bone turnover subdivided into markers of bone formation and bone disintegration, belong to the third group. During the choice of variables determined throughout the present work, this classification of bone biochemical markers was taken into account. So, the OC as a marker of bone formation and catalytic TRAP activity as a measure of osteoclastic activity were selected.

The results pointed to the significance of the serum TRAP activity in recognizing disturbances in the bone metabolism. Statistically significant differences in the catalytic activity of this enzyme between the two groups of examinees, as well as in relation to the reference range, support and confirm the data of Topić et al (13), while being in disaccord with the report of Petro (16). The results demonstrating significantly higher TRAP activity in the sera of patients with osteoporosis in relation to reference values are of utmost importance, opposite to the opinion of some authors that the determination of TRAP activity is a very expensive method for everyday practice. However, the specificity of this enzyme remains an unanswered question. Recent reports of Halleen et al (17) and Nenonen et al (18) pointed to TRAP 5b as a specific and sensitive marker of bone resorption because its activity was significantly increased in osteoporosis but also in osteopenia. This suggests that the determination of total TRAP catalytic activity and of its isoenzyme forms in the cases of decreasing bone mass could help to better understand this topic.

In our opinion, significant difference in the catalytic activity of TRAP between groups with osteoporosis and osteopenia observed in the present study is very important, especially having in mind the limitations of the T-score analysis. The analysis of individual TRAP values in the group with osteopenia showed that some 2/3 of examinees had values above the upper limit of the reference range. This result could be taken into account during the diagnostics of osteopenia.

Opposite to the data of Stavropoulou et al (14) obtained on experimental animals, no statistically significant differences were observed when the OC concentrations of either the group with osteoporosis or osteopenia were compared with the corresponding reference range. On the other hand, our results agree well with the data of Topić et al (13) who found increased OC levels in 30% of the patients with primary osteoporosis. However, in the present study, statistically significant differences in the OC level between the group with osteoporosis and that with osteopenia were observed ($p < 0.01$), pointing to the differences in bone turnover in these two groups of examinees.

On the basis of results obtained throughout the present study, it can be concluded that, together with other biochemical markers of bone turnover, determination of the catalytic TRAP activity and osteocalcin concentration in blood sera can not only improve the diagnostic potential of osteoporosis, but also be useful in its distinction from osteopenia.

TARTARAT-REZISTENTNA KISELA FOSFATAZA I OSTEOKALCIN KOD PACIJENATA SA OSTEOPOROZOM I OSTEOPENIJOM

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Kratak sadržaj: Mjerenjem katalitičke aktivnosti kisele fosfataze rezistentne na tartarat (TRKP) u serumu procjenjuje se osteoklastična aktivnost, pošto je koštane ćelije secerniraju tokom resorpcije. Osteokalcin je nekolagenski peptid koji učestvuje u procesu mineralizacije kosti. Korišten je u ranom prepoznavanju primarne osteoporoze. Cilj je bio da se utvrdi da li postoji razlika u katalitičkoj aktivnosti TRKP, kao i u serumskoj koncentraciji osteokalcina, u ispitanika sa osteoporozom i osteopenijom. Prva grupa (n=60) imala je T-scor mineralne gustine kosti (BMD) $\leq -2,3$, što se, prema standardima Svetske zdravstvene organizacije, definiše kao osteoporoza. Druga grupa (n=60) imala je T-scor između $-0,4$ i $-1,7$, što prema standardima Svetske zdravstvene organizacije odgovara smanjenoj koštanoj masi (osteopeniji). Katalitička aktivnost TRKP mjerena je kinetičkom metodom firme BIOMERIEUX (Njemačka), na analizatoru Mira Cobas plus (Njemačka), BMD određen je ultrazvučnom tehnikom, a koncentracija osteokalcina ECL tehnikom, firme ROCHE (Njemačka), na analizatoru Elecsys 2010 (Njemačka). Rezultati ukazuju na značajno povećanje katalitičke aktivnosti TRKP u serumu ispitanika sa osteoporozom, u odnosu na grupu sa osteopenijom ($p < 0,001$). Za osteokalcin je utvrđena statistički značajna razlika između dvije grupe ispitanika ($p < 0,01$). Smatramo da određivanje katalitičke aktivnosti TRKP u serumu, kao i koncentracije osteokalcina, uz druge biohemijske markere koštane pregradnje, može da unaprijedi dijagnostiku osteoporoze.

Ključne riječi: kiselina fosfataza rezistentna na tartarat, osteokalcin, osteoporoza, osteopenija

References

1. Kleerekoper M. Use of bone markers in the management of women with low bone density. *Meno Med* 2002; 10: 5–9.
2. Watts BN. Clinical Utility of Biochemical Markers of Bone Remodeling. *Clin Chem* 1999; 45: 8(B): 1359–68.
3. Kim SW, Park DJ, Park KS, Kim SY, Cho BY, Lee HK et al. Early Changes in Biochemical Markers of Bone Turnover Predict Bone Mineral Density Response to Antiresorptive Therapy in Korean Postmenopausal Women with Osteoporosis. *Endocrine J* 2005; 52 (6): 667–74.
4. Antonione R, Moro L. I marcatori di rimodellamento osseo. *Prospettive in pediatria* 2001; 31: 13–27.
5. Lepiarz-Rusek W, Kokocinska D. Early, non-invasive diagnostic of osteoporosis in men. *Pryegl Lek* 2005; 62 (7): 641–9.
6. Beastall GH. Evidence-based osteoporosis. *Jugoslav Med Biohem* 2004; 23: 213–19.
7. Chung YC, Ku CH, Chao TY, Yu JC, Chen MM, Lee SH. Tartrate-resistant acid phosphatase 5b activity is a useful bone marker for monitoring bone metastase in breast cancer patients after treatment. *Cancer Epidemiol Biomarkers Prev* 2006; 15 (3): 424–8.
8. Risteli J. Biomarkers of Bone Formation. *EMBODY*, Cambridge, UK, 2000.
9. Robins SP. Biochemical Markers of Bone Resorption. *EMBODY*, Cambridge, UK, 2000.
10. Emerk K. Bone markers and osteoporosis. *Jugoslav Med Biohem* 2004; 23: 221–8.
11. Majkić-Singh N. The role of the biochemical markers in detection of bone metabolism and diseases. *Jugoslav Med Biohem* 1997; 16: 185–196.
12. Majkić-Singh N. The biochemical markers of osteoporosis. Scientific Review. Serbian Scientific Society, Series: Medical and Biological Science, 2000; 27: 57–74.
13. Topić E, Primorac D, Janković S. Medicinsko-biokemijska dijagnostika u kliničkoj praksi. Medicinska Naklada, Zagreb, Hrvatska, 2004.
14. Stavropoulou A, Christopoulou GE, Anastassopoulos G, Panteliou SD, Lyritis GP, Spiliotis BE, et al. Alteration in serum leptin correlates with alterations in serum N-telopeptide of collagen type I and serum osteocalcin during the progression of osteoporosis in ovariectomized rats. *Clin Chem Lab Med* 2005; 43 (12): 1359–65.
15. Hodgson S, ed. Mayo Clinic o osteoporozii. Medicinska Naklada, Zagreb, Hrvatska, 2005.
16. Petro D. Biokemijski biljezi u dijagnostici metaboličkih koštanih bolesti. Osteoporozna 15. simpozij Hrvatskog društva medicinskih biohemičara, Zagreb, Hrvatska, 2004.
17. Halleen JM, Alatalo SL, Janckila AJ. Serum tartrate-resistant acid phosphatase 5b is a specific and sensitive marker of bone resorption. *J Bone Miner Res* 2000; 15 (1 Suppl): 524S.
18. Nenonen A, Cheng S, Ivaska KK, Alatalo SL, Lehtimäki T, Schmidt-Gayk H, et al. Serum TRACP 5b is a useful marker for monitoring alendronate treatment: comparison with other markers of bone turnover. *J Bone Miner Res* 2005; 20 (8): 1804–12.

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