RESEARCH ARTICLE

Matrix metalloproteinase-1 and -2 as markers of mineral bone disease in chronic kidney disease patients

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Abstract

Background: In the past few years, a distinct and multifactorial clinical entity called chronic kidney disease-mineral and bone disorder (CKD-MBD) that leads to decreased bone density and osteoporosis has been identified. The aim of this study was to assess the levels of the matrix metalloproteinase-1 and -2 (MMP-1 and MMP-2) in chronic kidney disease (CKD) patients of various disease stages in correlation to other bone turnover markers (BTM). This study is an initial investigative approach to a possible role of matrix metalloproteinases (MMPs) in the evaluation of bone disease in uremic patients.

Methods: We enrolled 60 patients at different stages of pre-dialysis CKD, 20 patients on hemodialysis (HD), and 20 age-matched healthy controls. Serum intact parathyroid hormone (iPTH), osteocalcin (OC), N-terminal propeptide of type I collagen (P1NP), and beta-C-terminal telopeptide of type I collagen (β-CTX), were measured by electrochemiluminescence on automatic analyzers. Serum MMP-1 and MMP-2 levels were estimated using a commercial enzyme-linked immunosorbent assay (ELISA). Serum levels of urea, creatinine, calcium, phosphorus, and alkaline phosphatase were estimated. Creatinine clearance (ClCr) was calculated using the traditional clearance formula based on a 24-hour urine collection.

Results: Serum iPTH, OC, P1NP, β-CTX concentrations were significantly higher ($p < 0.0001$) while ClCr was significantly lower ($p < 0.0001$) in CKD patients, as compared with those of healthy controls. A positive correlation was established between serum MMP-1 and OC levels ($r = 0.245$, $p = 0.014$), as well as with serum β-CTX levels ($r = 0.197$, $p = 0.048$), and a negative correlation between MMP-2 and OC ($r = -0.222$, $p = 0.025$).

Conclusions: In CKD patients MMP-1 serum levels may reflect increased bone turnover rates. HIPPOKRATIA 2017, 21(1): 25-31.

Keywords: Bone markers, matrix metalloproteinase-1, matrix metalloproteinase-2, chronic kidney disease, mineral bone disease

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Introduction

Chronic kidney disease (CKD) is related to abnormal calcium (Ca), phosphorus (P), vitamin D, and parathyroid hormone (PTH) serum levels, which are associated with impaired bone metabolism, leading to a specific type of osteopathy called Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD)[1]. CKD-MBD occurs when renal function is progressively reduced and includes a series of disturbances in bone modeling and remodeling, leading to bone disease and concomitant fractures[2]. During the progress of CKD, bone tissue is continuously influenced by pathogenic factors resulting in a progressive bone density loss. The main pathogenic factors that contribute to the development of CKD-MBD are impairment of calcitriol [$1,25(OH)_2D$] biosynthesis, hyperphosphatemia, and hyperparathyroidism. CKD-MBD related hypocalcemia is a consequence of low calcitriol levels and concomitant low enteric absorption of calcium. Also, the development of skeletal PTH resistance and the reduction of calcitriol receptors contribute to the progress of CKD-MBD. In CKD patients with creatinine clearance (ClCr) less than 60 ml/min, high PTH levels and high bone turnover rates are highly prevalent[3,4]. Fractures can occur at all stages of CKD. It has been repeatedly reported that patients with CKD have a higher risk of fracture as compared to age and bone density matched controls[5,6]. Therefore, monitoring bone metabolism using specific serum biochemical markers is considered important in CKD.

Various bone turnover markers (BTM) have been employed for the assessment and monitoring of CKD-MBD. Since PTH plays a central role (secondary hyperparathyroidism) in CKD-MBD, serum PTH levels have

been used for many years as the sole indicator of renal bone disease[3]. According to Kidney Disease Improving Global Outcomes (KDIGO) guidelines, the determination of PTH levels in CKD patients can provide solid evidence of underlying bone disease[7]. In the present study, the new bone markers deriving from collagen synthesis and breakdown were preferred to be investigated along with matrix metalloproteinases (MMPs) in CKD patients with renal bone disease.

Bone tissue synthetic activity is reflected by serum osteocalcin (OC) and N-terminal propeptide of type I collagen (P1NP) levels. OC is an index of osteoblast function and osteoid mineralization and is expressed mainly in the phase of bone formation while P1NP is derived from collagen type I and is considered as a useful indicator for monitoring and predicting bone disease[8,9]. On the other hand, beta-C-terminal telopeptide of type I collagen (β-CTX) and N-terminal telopeptide (NTX) are indicators of bone resorption. Elevated levels of β-CTX indicate increased bone turnover and kidney patients are at increased risk for rapid bone disease progression[9,10].

MMP-1 and MMP-2 are enzymes that degrade collagen type I, the major structural component of the bone organic matrix[11-13], and accordingly, their serum levels may provide an additional marker for the evaluation of CDK-MBD. Studies on osteoporotic women indicate that serum concentrations of MMP-1, MMP-2 and bone turnover are related[14,15].

Even though serum MMP-1 and MMP-2 levels have been studied in CKD patients as indicators of inflammation[16-18], their significance in CKD-MBD remains to be elucidated.

Subjects and Methods

Subjects

In this prospective case-control study conducted from April 2012 to December 2015, serum samples derived from 80 enrolled adult patients who attended the AHEPA University Hospital outpatient clinics for CKD treatment, were collected. Patients at various stages of CDK and with various forms of the related bone disorders were included. In addition, 20 healthy age-matched individuals, served as control subjects. The causes of renal disease were the following: diabetic nephropathy (n =29), chronic glomerulonephritis (n =36), nephrosclerosis (n =6), polycystic kidney disease (n =5), arterial hypertension (n =2), and unknown (n =2). Patients treated with corticosteroids or non-steroidal anti-inflammatory analgesics, along with patients with diabetes mellitus treated with insulin and patients diagnosed with any form of cancer where excluded from the study.

The patients were divided into four groups according to the severity of renal disease and ClCr. Each group included 20 subjects. The first group CKD2 included subjects with estimated ClCr 60-90 ml/min (stage 2). The second group CKD3 included patients with ClCr 30–60 ml/min (stage 3), the third group CKD4 included patients with ClCr 15–30 ml/min (stage 4), and the fourth group CKD5 included 20 patients under hemodialysis (HD) with ClCr <15 ml/min (stage 5-HD). In patients undergoing hemodialysis, the mean time of HD treatment was 61.4 ± 12.1 months. Dialysis was performed three times a week and lasted four hours with low flux polysulfone or polyacrylonitrile dialyzers (Fresenius Medical Care, Bad Homburg, Germany).

The study was approved by the Bioethics Committee of the Faculty of Medicine of the Aristotle University of Thessaloniki (decision No: A47, date: 01/09/2010) and informed consent for participation in the study was obtained from every patient.

Blood sampling and laboratory tests

Venous blood samples were obtained from all patients and after clotting, serum was separated and analyzed immediately or stored in aliquots at -70° C upon analysis. Blood samples from HD patients were taken directly from the arteriovenous fistula immediately before the beginning of the HD session.

Conventional clinical biochemistry was performed by colorimetry. Specific bone disease markers and hormones were analyzed by electrochemiluminescence immunoassay on automated analyzers. OC and intact parathyroid hormone (iPTH) were measured on the Modular Analytics E170 analyzer (Roche Diagnostics GmbH, Mannheim, Germany), and total P1NP and β-CTX were measured on the Elecsys 2010 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). ClCr is used to estimate the glomerular filtration rate (GFR) and is calculated by determining creatinine concentration in 24-hour urine collection and blood using the following equation:

ClCr (ml/min) = [urine creatinine (mg/dl) x urine volume (ml/min)] / [serum creatinine (mg/dl) x T*]
* T =24 hours x 60 min =1440 min

Serum levels of MMP-1 and MMP-2 were estimated using a commercial enzyme-linked immunosorbent assay (ELISA-RayBiotech, Norcross, USA). The immunoassay kits for MMP-1 and MMP-2 recognize both pro and active forms of MMPs.

Statistical analysis

Statistical analysis was performed using the IBM SPSS Statistics for Windows software, version 20.0 (IBM SPSS, IBM Corp., Armonk, NY, USA) and JMP 8.0 (SAS Inst., Cary, NC). Data were expressed as means ± standard deviation. One-way ANOVA analysis was used for comparing quantitative measurements of the study groups. Mann-Whitney U test and non-parametric Kruskal-Wallis test were used to evaluate the statistical significance of the differences between the groups. The data in most cases did not follow the normal distribution, and for this reason, the correlations were evaluated using the Spearman's coefficient. The latter was also applied, because of the sample size per group. A p value less than 0.05 was considered statistically significant.

Results

The descriptive data and characteristics of the studied CKD patients are summarized in Table 1. Significant differences ($p < 0.0001$ or $p < 0.05$) were noted for iPTH, OC, P1NP, and β-CTX levels among patients with different stages of CKD compared to controls. As ClCr is reduced, a gradual increase of the bone indicators is observed ($p < 0.0001$). The levels of iPTH, OC, P1NP, and β-CTX were significantly higher ($p < 0.0001$) in the dialysis patients (Table 2).

MMP-1 serum levels showed a weak gradual increase with the deterioration of kidney function in CKD patients and had the highest values in hemodialysis patients, which differed significantly from the healthy individuals ($p = 0.0468$). On the contrary, MMP-2 serum levels showed statistically significantly lower levels ($p = 0.0074$, $p = 0.0098$, $p = 0.0340$ and $p = 0.0154$, respectively) between all other groups and hemodialysis patients (Table 2).

Positive correlations between iPTH levels and the others bone markers (OC, P1NP and β-CTX) were established while significant positive correlations were also observed among all the bone disease markers tested (Table 3). It should also be emphasized that we found negative correlations between ClCr and bone markers in the studied groups. All the biochemical bone markers analyzed were significantly negatively correlated with ClCr (Table 4).

MMP-1 showed a significant positive correlation with OC and β-CTX whereas a significant negative correlation was found between MMP-2 and OC (Table 3).

No further association was found between MMP-1 and MMP-2 and the biochemical bone parameters in all the studied groups.

Discussion

Bone indicators & metalloproteinases in CKD

In an attempt to evaluate a possible diagnostic significance of serum MMP-1 and MMP-2 levels considering CKD-MBD monitoring, the levels of MMPs were estimated in the serum of CKD patients in different disease stages along with the levels of iPTH, OC, P1NP, and β-CTX.

The serum levels of iPTH, OC, P1NP, and β-CTX

Table 1: Characteristics and studied parameters of the 60 patients at different stages of pre-dialysis chronic kidney disease (CKD), 20 patients on hemodialysis (HD) and 20 matched healthy controls that were included in the study

	Controls (A)	CKD2 (stage 2) (B)	CKD3 (stage 3) (C)	CKD4 (stage 4) (D)	CKD5 (stage 5-HD) (E)
Males/females	8/12	11/9	10/10	11/9	14/6
Age, years	67.65 ± 3.99	66.50 ± 6.55	69.70 ± 5.79	65.45 ± 8.83	55.30 ± 8.04
ClCr (ml/min)	107.04 ± 10.45	76.87 ± 10.79 A-B p <0.0001*	47.17 ± 7.88 A-C p <0.0001* B-C p <0.0001*	22.73 ± 5.80 A-D p <0.0001* B-D p <0.0001* C-D p <0.0001*	<15
U (mg/dl)	34.4 ± 8.42	48.25 ± 18.83 A-B p =0.0033*	74.25 ± 41.10 A-C p <0.0001* B-C p =0.0015*	100.75 ± 47.89 A-D p <0.0001* B-D p =0.0003*	154.95 ± 44.01 A-E p <0.0001* B-E p <0.0001* C-E p <0.0001* D-E p =0.0022*
Cr (mg/dl)	0.58 ± 0.05	1.17 ± 0.40 A-B p =0.0294*	1.78 ± 0.66 A-C p =0.0015* B-C p =0.0009*	3.33 ± 2.29 A-D p =0.0010* B-D p <0.0001* C-D p =0.0161*	9.05 ± 1.87 A-E p <0.0001* B-E p <0.0001* C-E p <0.0001* D-E p <0.0001*
Ca (mg/dl)	9.48 ± 0.68	9.25 ± 0.37	9.43 ± 0.66	8.90 ± 0.54 A-D p =0.0058* B-D p =0.0385* C-D p =0.0049*	8.50 ± 0.93 A-E p =0.0011* B-E p =0.0098* C-E p =0.0007*
P (mg/dl)	3.98 ± 0.8	3.27 ± 0.56 A-B p =0.0040*	3.38 ± 0.55	4.34 ± 1.22 B-D p =0.0004* C-D p =0.0086*	6.22 ± 1.39 A-E p <0.0001* B-E p <0.0001* C-E p <0.0001* D-E p <0.0001*
Alp (mg/dl)	70.66 ± 4.00	69.9 ± 33.75	82.6 ± 30.78 A-C p =0.0133* B-C p =0.0453*	89.45 ± 61.60	84.65 ± 25.52 A-E p =0.0149* B-E p =0.0326*

CKD: chronic kidney disease, HD: hemodialysis, ClCr: creatinine clearance, U: urea, Cr: creatinine Ca: calcium, P: phosphorus, Alp: alkaline phosphatase. One-way ANOVA analysis was used for comparing quantitative measurements of the study groups. The asterisk (*) indicates statistically significant differences between the studied parameters.

Table 2: Serum levels of intact parathyroid hormone (iPTH), osteocalcin (OC), N-terminal propeptide of type I collagen (P1NP), β-C-terminal telopeptide of type I collagen (β-CTX) and matrix metalloproteinases (MMPs) in the different stages of pre-dialysis chronic kidney disease (CKD) and in healthy controls.

	Controls (A)	CKD2 (stage 2) (B)	CKD3 (stage 3) (C)	CKD4 (stage 4) (D)	CKD 5 (stage 5-HD) (E)
iPTH (pmol/l)	4.96 ± 1.40	7.93 ± 4.89	9.51 ± 2.65 A-C p <0.0001* B-C p =0.0337*	15.30 ± 11.89 A-D p <0.0001* B-D p =0.0115*	34.32 ± 22.18 A-E p <0.0001* B-E p <0.0001* C-E p <0.0001* D-E p =0.0007*
OC (ng/ml)	27.7 ± 10.58	20.35 ± 9.43 A-B p =0.0154*	36.40 ± 18.07 B-C p =0.0003*	61.77 ± 53.13 A-D p =0.0049* B-D p =0.0002*	199.75 ± 87.26 A-E p <0.0001* B-E p <0.0001* C-E p <0.0001* D-E p <0.0001*
β-CTX (ng/ml)	0.31 ± 0.11	0.32 ± 0.17	0.68 ± 0.35 A-C p =0.0008* B-C p =0.0010*	0.79 ± 0.63 A-D p =0.0077* B-D p =0.0069*	2.22 ± 1.08 A-E p <0.0001* B-E p <0.0001* C-E p <0.0001* D-E p <0.0001*
P1NP (ng/ml)	60.48 ± 30.87	53.33 ± 27.14	82.66 ± 44.62 A-C p =0.0468* B-C p =0.0124*	99.66 ± 65.69 A-D p =0.0167* B-D p =0.0071*	262.94 ± 131.78 A-E p <0.0001* B-E p <0.0001* C-E p <0.0001* D-E p <0.0001*
MMP-1 (pg/ml)	2169.69 ± 1746.56	2731.1 ± 1756.06	2798.19 ± 1589.14	3012.28 ± 2732.68	3528.9 ± 2516.01 A-E p =0.0468*
MMP-2 (ng/ml)	135.01 ± 72.16	134.01 ± 59.15	139.36 ± 103.03	143.15 ± 62.39	98.07 ± 54.99 A-E p =0.0074* B-E p =0.0098* C-E p =0.0340* D-E p =0.0154*

CKD: chronic kidney disease, HD: hemodialysis, iPTH: intact parathyroid hormone, OC: osteocalcin, β-CTX: β-C-terminal telopeptide of type I collagen, P1NP: N-terminal propeptide of type I collagen, MMP-1: metalloproteinase-1, MMP-2: metalloproteinase-2. One-way ANOVA analysis was used for comparing quantitative measurements of the study groups. The asterisk (*) indicates statistically significant relationships between the studied parameters.

Table 3: Correlations between bone biochemical parameters and the matrix metalloproteinases (MMPs) in the studied groups.

	iPTH (pmol/l)	OC (ng/ml)	β-CTX(ng/ml)	P1NP (ng/ml)	MMP-1 (pg/ml)	MMP-2 (ng/ml)
iPTH (pmol/l)	––	rho =0.674 p <0.0001*	rho =0.743 p <0.0001*	rho =0.657 p <0.0001*	rho =0.168 p =0.092	rho =-0.129 p =0.200
OC (ng/ml)		––	rho =0.809 p <0.0001*	rho =0.756 p <0.0001*	rho =0.245 p =0.014*	rho =-0.222 p =0.025*
β-CTX (ng/ml)			––	rho =0.890 p <0.0001*	rho =0.197 p =0.048*	rho =-0.187 p =0.061
P1NP (ng/ml)				––	rho =0.122 p =0.224	rho =-0.147 p =0.142
MMP-1 (pg/ml)					––	rho =0.206 p =0.039*

iPTH: intact parathyroid hormone, OC: osteocalcin, β-CTX: β- C-terminal telopeptide of type I collagen, P1NP: N-terminal propeptide of type I collagen, MMP-1: metalloproteinase-1, MMP-2: metalloproteinase-2. The asterisk (*) indicates the statistically significant correlation between the studied parameters.

Table 4: Correlations between clearance creatinine, bone biochemical parameters and the matrix metalloproteinases (MMPs) in the studied groups [excluding the hemodialysis patients (CKD 5-HD$^{\lozenge}$)].

	ClCr (ml/min)	iPTH (pmol/l)	OC (ng/ml)	β-CTX (ng/ml)	P1NP (ng/ml)	MMP-1 (pg/ml)	MMP-2 (ng/ml)
ClCr (ml/min)	––	rho =-0.574 p <0.0001*	rho =-0.391 p =0.0003*	rho =-0.389 p =0.0004*	rho =-0.345 p =0.0017*	rho =-0.146 p =0.193	rho =-0.007 p =0.949

ClCr: creatinine clearance, iPTH: intact parathyroid hormone, OC: osteocalcin, β-CTX: β-C-terminal telopeptide of type I collagen, P1NP: N-terminal propeptide of type I collagen, MMP-1: metalloproteinase-1, MMP-2: metalloproteinase-2. The asterisk indicates (*) the statistically significant correlation between the studied parameters. $^{\lozenge}$: The CKD5 (stage5-HD), the group of dialysis patients was not included because it is difficult to measure ClCr in patients who hardly produce urine.

of all the studied groups indicated a significant increase with the progression of the disease ($p < 0.0001$ or $p < 0.05$). These results support the notion that progressive deterioration of renal function leads to an increased bone turnover. This view is also underlined by the significant positive correlations between the above markers (Table 3). On the other hand, the significant negative correlation of these markers with ClCr confirms the influence of low glomerular function in bone metabolism (Table 4).

The results of the present study indicate that serum iPTH levels correlate significantly with the serum levels of OC, β-CTX, and P1NP. Moreover, significant positive correlations between the bone markers (OC, β-CTX, P1NP) were observed (Table 3). These observations indicate that iPTH serum levels increase as the different stages of renal disease progress together with the other bone markers reaching the highest values in the dialysis patients. Increased iPTH levels demonstrate that high bone turnover is present in CKD patients, which is related to uremia. Accordingly, Takano et al reported that serum OC levels in hemodialysis patients correlate with the serum levels of iPTH and that patients with high serum PTH had significantly higher levels of the bone markers, as compared with patients with normal or low PTH values[19]. In a recent study that included CKD stages 3 to 5 patients, serum levels of iPTH, OC, P1NP, and β-CTX were found to increase significantly with the stage of the disease[20]. Other researchers also reported that the levels of serum bone markers gradually increase with the deterioration of renal function[21]. It should also be noted that although OC and β-CTX serum levels, may represent opposing processes in the bone tissue, they are both elevated in the case of accelerated bone remodeling. Several studies have demonstrated that both indicators of bone formation and resorption can be reliable criteria for assessing the risk for bone loss in kidney patients. Tsuchida et al showed increased values of bone formation markers, indicative of increased bone remodeling while Okuno et al found that elevated levels of serum β-CTX and OC are associated with rapid bone loss, as defined using bone density assessment techniques (DEXA: Dual Energy X-ray Absorptiometry)[22,23]. Our results confirm the findings of the above studies and corroborate the view that these biomarkers of bone metabolism might be clinically significant for the evaluation of renal bone disease and could be used for the follow-up of bone mineral disease in advanced stages of CKD and HD.

The assessment of bone mineral density (BMD) using DEXA is controversial for bone loss in CKD patients[24] and modifications in bone tissue need time to be traceable. On the other hand, quantitative computed tomography (QCT) is expensive, and patients are exposed to higher levels of radiation[24]. Bone biopsy is the gold standard method for the diagnosis and classification of renal osteodystrophy[25]; however, it is an invasive technique and cannot be routinely performed. Thus, the use of biochemical bone markers can be clinically helpful to monitor bone status in renal bone disease in a comparatively faster affordable way, without radiation exposure. In our study, increased levels of bone markers in patients with CKD underline their predictive value and their importance in the assessment and follow-up of renal bone disease.

MMPs are a family of extracellular matrix (ECM) degrading enzymes that play an important role in the homeostasis of the ECM and hence to development, growth, and regeneration of tissues[26]. MMP-1 and MMP-2 play an important role in bone collagen remodeling and may be involved in pathological conditions affecting bone metabolism[26-28]. The expression of MMPs in bone and cartilage cells during bone development, as investigated in knock-out mice models and human genetic diseases, has demonstrated prominent importance of those MMPs[29].

In the present study, serum MMP-1 levels were found significantly increased only in dialysis patients compared to healthy subjects ($p = 0.0468$). However, an upward trend of MMP-1 with the stage of the disease was observed (Table 2). MMP-1 serum levels correlated positively with OC and β-CTX and seem to interfere actively in bone turnover. Despite the noted increase of MMP-1 and the concomitant decrease of MMP-2 in HD patients a significant positive correlation between MMP-1 and MMP-2 was also observed (Table 3). Even though there seems to be a statistical correlation between the above parameters, this finding may represent a spurious relationship in which these two variables are not causally and logically related to each other; yet it may be wrongly inferred that they are. Another explanation for this statistical paradox could be that the latter may partly be attributed to the small sample size of the current study.

MMP-2 serum levels were found significantly decreased in HD patients and showed a negative correlation with OC (Table 3). This finding might suggest that MMP-2 is indirectly involved in bone degradation. Elevated levels of OC indicate increasing bone formation, and the decrease of MMP-2 may be associated with a reduction of the bone resorption. This result reveals a connection between the bone response and the probable influence of the collagenolytic activity of MMP-2 on bone metabolism. Possibly an unknown mechanism that acts in HD patients inhibits the expression of MMP-2 and upregulates the bone degradation. Thus, an unexpected reduction in MMP-2 levels in HD patients is observed.

In the past, serum levels of MMPs have been studied in the context of osteoporosis. Nonetheless, to the best of our knowledge, this is the first study that compares serum MMP-1 and MMP -2 levels with other bone markers in CKD patients.

MMP-2 is involved with bone metabolism via its effect on osteoclast and osteoblast activity and proliferation. However, the mechanisms are still not fully clarified[11]. The fact that bisphosphonates can inhibit bone resorption in secondary osteoporosis patients by decreasing MMP-2 and TRACP-5b[30] which is a marker indicative of osteoclast activity, strengthens the aspect that MMP-2 has an important role in bone resorption. MMP-2 is pro-

duced in the skeletal tissue and can be released into the circulation in diseases related with a high bone turnover. MMP-2 serum concentrations have been reported to be significantly higher in women with osteoporosis as compared to healthy age-matched controls, with a significant correlation of MMP-2 with bone turnover markers[14,15]. In contrast to these studies, we observed a decrease in the expression of MMP-2 in the HD patients. This documented difference of our study may reflect alterations in the pathophysiological background between osteoporosis and renal bone disease. MMPs are expressed in the human glomerulus and play a prominent role in glomerular inflammatory diseases[17,29]. In addition, it should be emphasized that MMPs are also expressed in bone, cleave type I collagen and play an important role in bone remodeling[12,13]. However, their use as possible complementary bone indices has not been investigated.

Osteoporosis and renal osteodystrophy are present in CKD-MBD. Bone abnormalities are found in the majority of patients at different CKD stages and almost in all CKD patients on dialysis treatment[31]. The data of the present study indicate significant correlations between bone markers and MMP-1 (Table 3) suggesting an association between bone turnover and MMP-1 serum levels.

A number of factors, such as toxic uremic environment, increased levels of cytokines, and oxidative stress, lead to a persistent inflammatory condition in CKD and particularly in end-stage renal disease[32,33]. These factors and the dialysis procedure per se could affect the serum concentrations of these biomarkers.

The present study has certain limitations. Firstly, the number of individuals in the different patient groups is small. Secondly, we did not assess the inflammatory state in CKD patients. The latter, via the upregulation of cytokines, may have contributed to abnormal bone turnover. The decrease of MMP-2 in dialysis patients in our study is conflicting. The additional detection of inflammatory markers such as interleukin-6 and C-reactive protein as well as the determination of the specific endogenous tissue inhibitors of metalloproteinases (TIMPs) might have been helpful in clarifying this finding. Eleftheriadis et al have shown that chronic inflammation, especially in HD patients, could have a negative impact on bone turnover, possibly by the increase of IL-6[34]. Also, it has been reported that MMP-1 can be produced by other cell types, such as the fibroblast, and interestingly it is upregulated in case of inflammation[35]. A third limitation of the study is the fact that OC, β-CTX, and P1NP are renally excreted, and consequently their increased levels with the progression of CKD cannot be attributed exclusively to increased bone turnover. However, these biomarkers could be considered as important promising indicators even for uremic patients. Although cleared by the kidney, the high concentrations of these markers reflect an increased metabolic activity of the bone caused by the secondary hyperparathyroidism[36]. The positive correlation between OC, β-CTX, P1NP, and iPTH supports and strengthens the use of these serum markers to estimate bone turnover in CKD. Despite the oxidation and the inflammation, PTH secretion is hardly suppressed. One of the largest and most recent studies confirmed that PTH is currently the most useful marker for bone turnover in CKD[37]. The impact of diminished clearance of these biomarkers and their importance in the clinical setting must always be considered in CKD[37,38]. Nevertheless, it must also be noted that iPTH cannot predict the bone turnover status in one-third of HD patients[39].

In conclusion, the data presented here indicate that MMP-1 levels are elevated with the progression of CKD-MBD, and correlate with other bone markers. Considering MMP-2 serum levels a negative correlation with serum OC levels was found. This observation may indirectly implicate MMP-2 in bone metabolism. MMPs serum levels may reflect bone remodeling rate in these patients since they seem to correlate with the bone markers. Further studies are needed to elucidate the pathophysiological mechanisms reflected by these observations.

Conflict of interest

The authors declare no conflict of interest.

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