NEW BIOCHEMICAL SERUM MARKERS OF BONE TURNOVER IN RENAL OSTEODYSTROPHY

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Abstract

Renal osteodystrophy is a multifactorial disorder of bone remodelling that develops in patients with chronic renal failure. During the last few years numerous biochemical markers of bone turnover have been proposed for the non-invasive diagnosis of renal osteodystrophy.

Several enzymes and matrix proteins produced by osteoblasts and osteoclasts, including collagen type I degradation products, have been recognized as circulating biochemical markers of both bone formation and bone resorption process.

The aim of this article was to present and estimate the clinical utility of new serum markers of bone metabolism like bone specific alkaline phosphatase (bAP), procollagen type I extension peptides (PICP/PINP), osteocalcin (Oc), tartrate-resistant acid phosphatase (TRAP), procollagen type I crosslinked carboxyterminal telopeptide (ICTP), pyridinoline (PYR), deoxypyridinoline (DPD), C- or N-terminal telopeptides of type I collagen (CTx, NTx) and other potential markers in diagnosis of renal osteodystrophy in patients with chronic renal failure.

Key words: renal osteodystrophy, bone formation markers, bone resorption markers

Introduction

Mineral metabolism is under control of the kidneys, intestine, parathyroid glands and bone. The kidney plays a critical role in mineral homeostasis regulation and, therefore, renal disease exerts widespread effects on the skeleton and soft tissues.

Renal osteodystrophy is the term used to describe abnormalities of the skeleton and soft tissues in renal failure. The complex disorder of the skeleton is an important cause of morbidity and decreased quality of life [1,2]. Phosphate retention, hypocalcaemia, insufficient production of 1,25 (OH)2D3 and the resistance of bone tissue to the action of parathyroid hormone (PTH) are the main factors that cause metabolic disturbances and lead to the development of renal osteodystrophy. Aluminium poisoning, a consequence of aluminium retention and metabolic acidosis may intensify bone disorders [3].

Recent data suggest that the bone morphogenetic proteins (BMP) deficiency and decreased osteoblast's differentiation may play a part in the pathophysiology of renal osteodystrophy [4].

Bone is a dynamic tissue which is continuously remodelled [5]. In pathologic conditions, such as renal osteodystrophy this process may be accelerated or down-regulated, so renal osteodystrophy is not a uniform disease and is often classified according to the state of bone turnover [1,2,5]:

1. High turnover bone disease (HTBD) results from secondary hyperparathyroidism. Severe osteitis fibrosa and moderate hyperparathyroidism can be distinguished.

2. Low turnover bone diseases (LTBD) are represented by:

    - Osteomalacia: characterized by low bone formation and a defect in bone matrix mineralization which occurs after cessation of bone growth. In ostomalacia unmineralized bone collagen is accumulated

    - Adynamic bone disease: characterized by reduced bone turnover and normal or decreased amount of osteoid

3. Mixed forms, where elements resulting from hyperparathroidism occur in combination with elements of osteomalacia [3].

In recent years, several enzymes and matrix proteins synthesized by osteoblasts and protein fragments released after bone matrix breakdown have been proposed as markers of renal osteodystrophy (Table 1) [6,7]. Non-invasive diagnosis includes markers of bone formation such as bone specific alkaline phosphatase (bAP), osteocalcin (Oc), procollagen type I carboxy-terminal propeptide (PICP) and markers of bone resorption as pyridinoline, deoxypyridinoline, tartrate-resistant acid phosphatase (TRAP), procollagen type I crosslinked C-terminal telopeptide (ICTP) and C- or N-terminal telopeptides of type I collagen [8]. New biochemical serum markers of bone remodeling in renal osteodystrophy are summarized in Table 1. Circadian rhythms, diet, age, gender, menopause, liver function, clearance rates, all have an influence on the interpretation of serum concentrations of these markers in chronic renal failure. Further studies are needed to find the ideal biochemical marker for monitoring bone turnover in uraemia [6,7].

Table 1 Biochemical markers of bone remodeling in renal osteodystrophy

Markers of bone formation	Markers of bone resorption	Other potential markers
Total alkaline phosphatase (tAP)	Tartrate-resistant acid phosphatase (TRAP)	Cytokines (IL-1, IL-6, IL-11, TNF-?, TGF-?, IFN-?)
Bone specific alkaline phosphatase (bAP)	Procollagen type I cross linked carboxy terminal telopeptide (ICTP)	Osteoprotegerin (OPG)
Osteocalcin (Oc)		?2-microglobulin (?2m)
	Pyridinoline (PYD)	
Procollagen type I C- and N-terminal propeptides (PICP and PINP)	Deoxypyridinoline (DPD)	Advanced glycation end products (AGE)
	Cross-linked C- and N-terminal telopeptides of type I collagen (CTx and NTx)	Bone sialoprotein (BSP)

Biochemical serum markers of bone formation

Alkaline phosphatase (AP) and bone-specific alkaline phosphatase (bAP)

AP is a glycosylated protein produced by different organs: liver, bone, kidney, intestine and placenta [6,7]. One single gene codes group of AP that consists of liver, bone and kidney and respective isoenzymes differ only by post-transcriptional glycosylation [9]. The bone isoenzyme is produced by osteoblasts and osteoblast precursors [6,7,10]. Plasma activity of bAP is not modified by variations in renal function because it is neither dialyzable nor filtrable by the kidneys [10]. bAP has the highest physiological activity in childhood and particularly during puberty [9,10].

In adult patients on haemodialysis, bAP concentration > 20 ng/ml had sensitivity and specificity of 100% for the diagnosis of HTBD and positive predictive value of 84% [6,7,10]. When bAP value is over 20 ng/ml and serum PTH level is above 200 pg/ml, suggesting secondary hyperparathyroidism, then the sensitivity of bAP decreases to 56%, specificity to 92% and positive predictive value for the diagnosis of HTBD increases from 84% to 94% [6,7,10]. Many patients have increased PTH values over 200 pg/ml without elevated bAP [10]. Osteomalacia, characterized by hypophosphatasia, in

which the enzyme is lacking, suggests that alkaline phosphatase plays a role in the mineralization of newly formed bone [9].

Couttenye et al demonstrated that low (<27 U/l) level of bAP and low (<150 pg/ml) level of intact PTH (iPTH) are good markers of adynamic bone disease [7]. Results by Coen et al showed that the determination of iPTH and bAP may be adequate in the discrimination of bone histological patterns of low turnover osteodystrophy [11]. The results of their study in patients on haemodialysis, who underwent bone biopsy, demonstrated that plasma bAP concentration lower than 12,9 ng/ml had a sensitivity of 100 % and specificity of 94 % for the diagnosis of LTBD [10,11].

Nakai et al reported that if elevated serum levels of AP and carboxy-terminal parathyroid hormone (c-PTH) are found in haemodialysis patients, secondary hyperparathyroidism should be treated in order to prevent a decrease in bone mineral density, especially in patients with glomerulonephritis [12].

Osteocalcin (Oc)

Osteocalcin represents one of the most abundant non-collagenous proteins of the bone matrix but is also present in dentine and calcified cartilage [6,7,10]. Osteocalcin is synthesized by osteoblasts under the control of $1,25(OH)_2D_3$ [6,7,10,13]. Three residues of vitamin K-dependent amino acid, ã-carboxyglutamic acid (Gla), facilitate the binding of osteocalcin to hydroxyapatite in bone [13]. Circulating intact osteocalcin represents 26% of total osteocalcin in patients with chronic renal failure [10]. Plasma Oc has poor stability and is removed by the kidneys [6,7]. The blood osteocalcin concentration is used as one of the sensitive markers of bone formation and reflects the underlying bone histology in renal osteodystrophy [13]. Urenþa et al showed that the plasma levels of Oc demonstrated good sensitivity allowing the distinction between patients with hyperparathyroidism and those with normal or low bone turnover. However, it has low diagnostic sensitivity in discrimination between patients with adynamic bone disease and with normal bone turnover [10]. Bervoets et al demonstrated that Oc, AP, bAP and serum calcium levels are useful in the diagnosis of adynamic bone disease, normal bone and osteomalacia in predialysis patients with end-stage renal failure [14].

Procollagen type I carboxy-terminal propeptide (PICP) and procollagen type I amino-terminal propeptide (PINP)

PICP and PINP are by-products of type I collagen synthesis. Both are metabolised by the liver. They may be incorporated into the bone matrix. The concentrations of PICP and PINP increase with increased turnover of non-skeletal collagen (e.g. skin, muscle) [9]. PICP plasma concentration is not altered by renal failure because it is degraded by the liver through the mannose-6-phosphate receptor [10,15]. Because of that, the serum level of PICP is independent of renal function and its concentration reflects dynamic parameters of bone metabolism in adults with predialytic renal failure [16]. PICP is produced by osteoblasts during the process of bone formation and has been used as serum marker of bone formation [6,7,10]. Significantly increased plasma PICP levels were observed in nondialyzed patients with chronic renal failure [10]. However this increase does not correlate with static histomorphometric parameters measured on biopsy specimens nor with other humoral markers of bone turnover [6,7]. The results by Polak-Jonkisz et al indicate that the levels of PICP and ICTP should be routinely monitored as specific biochemical markers of bone structure in children with chronic renal insufficiency in the predialysis period, because clinical symptoms related to renal osteodystrophy usually appear late in the course of disease and bone turnover alterations

are present early during the disease process [15]. Moreover, they demonstrated a positive correlation between PTH and PICP in patients with elevated serum concentrations of PTH and weak positive relationship between PICP and Oc that was confirmed in patients with symptoms of parathyroid hyperactivity [15]. On the contrary observations of others have not demonstrated any great clinical value of plasma PICP in the diagnosis of bone remodelling in haemodialysis patients [10]. Nowak et al found significant correlation between iPTH and PINP, and iPTH and TRAP 5b that indicated the usefulness of these markers of bone turnover ****in dialysed patients [17].

Biochemical markers of bone resorption

Tartrate-resistant acid phosphatase (TRAP)

TRAP appears at least in 5 isoforms, which origin from the prostate, erythrocytes, platelets, bone, spleen and macrophages. All acid phosphatases are inhibited by tartrate, except subform 5b that is characteristic for osteoclasts. TRAP exists in big amounts in the scopulated edge of osteoclast and is released during bone resorption. The subform 5b differs from 5a because of the presence of sialic acid residues connected with the particle.

The clinical usefulness of TRAP as a marker of bone resorption results from the high increase of its concentration in serum in the course of this process. TRAP 5b is responsible for increased tartrate-resistant acid phosphatase activity in renal osteodystrophy. It is also associated with osteoclastic activity in non-uraemic patients.

Correlation between serum TRAP and bAP have been observed. Recently it was shown that serum TRAP activity is related to number of osteoclasts and the percentage of eroded bone surface. Finally, TRAP correlates with serum iPTH and total AP [10].

Studies showed significant changes in TRAP 5b levels in a very early stage of renal osteodystrophy. The results suggest that it might be an important marker of bone resorption in haemodialysis patients [18].

Others have found that isoform 5a was normal and isoform 5b was elevated in end stage renal disease. Increased levels of TRAP in ERSD were due to osteoclastic 5b activity and related to bone turnover [19].

The value of serum TRAP measurement in patients with renal osteodystrophy still remains to be established and more sensitive and simple methods are currently being evaluated.

Procollagen type I crosslinked carboxyterminal telopeptide (ICTP)

Type I collagen is the major component of extracellular bone matrix where it forms about 90% of the organic matrix. ICTP is the carboxyterminal telopeptide region of type I collagen, joined via trivalent cross-links and released during the degradation of mature type I collagen during bone resorption. ICTP is significantly increased in patients with disorders of bone metabolism. Results of several clinical studies indicated that ICTP is a valuable marker of bone resorption and could serve as a useful marker for haemodialysis patients.

In one Polish study it was shown that serum levels of ICTP in children with chronic renal failure were twice as high as in a control group, suggesting decreased renal clearance of ICTP [15]. In low turnover osteodystrophy in haemodialysis patients, others observed that elevated levels of ICTP correlated with iPTH, AP, bAP and

deoxypyridinoline concentrations. They proposed ICTP as an important biochemical marker of bone turnover in renal osteodystrophy [20]. However, more recent clinical investigations have not supported the clinical utility of ICTP measurement in chronic renal failure. For example, Ferreira observed, in dialysis patients, a significant correlation between serum levels of ICTP and any of the numerous parameters. He suggested that ICTP is not a sensitive marker of bone metabolism in uraemic patients [7].

Pyridinoline (PYR) and deoxypyridinoline (DPD)

Pyridinoline cross-links of collagen exist in two chemical forms namely pyridinoline (PYR) and deoxypyridinoline (DPD). These molecules are markers of type I and II collagen degradation and are ideal parameters of bone resorption in several metabolic bone diseases.

Deoxypyridinoline is more specific for bone, pyridinoline is also found in articular cartilage and in soft tissues. Type I collagen from bone is unique in that the ratio of PYR to DPD is 3.5:1 compared to 10:1 found in most connective tissues.

During the process of bone resorption by osteoclast-derived enzymes, PYD and DPD are released into the blood in free form and as a part of peptides and later excreted in the urine [10]. Serum levels of PYD and DPD are low or undetectable in healthy subjects; therefore these markers are commonly detected in the urine. PYD and DPD are increased in patients with severe renal failure.

Ferreira, for the first time, demonstrated that serum PYR can be measured in dialysis patients who have markedly higher serum PYR levels than normal individuals. The highest values of serum PYR were observed in patients with the highest rate of bone resorption [7].

In other studies, authors have observed that circulating PYD and DPD levels were 50 to 100 times higher in haemodialysed patients than in controls. It is suggested that high serum PYR and DPD levels are generally associated with high turnover bone disease including chronic renal failure [10].

It has also been shown that serum levels of pyridinium crosslinks are increased in haemodialysis patients. Moreover, PYR and DPD correlated with other parameters of bone metabolism [21]. One study demonstrated that serum PYR correlated better with bone mineral density (BMD) than PTH in haemodialysis patients. In this study elevated serum levels of serum pyridinium crosslinks indicated negative influence on BMD. This may suggest a cause for increased fracture risk observed in chronically dialyzed patients [22].

In other studies of low turnover osteodystrophy in haemodialysis patients, significant correlation between intact PTH and DPD was observed. The results indicated that DPD also correlate with most of the histomorphometric and histodynamic parameters evaluated in biopsy specimens [11].

It may be concluded that serum levels of PYR and DPD in patients with chronic renal failure may be useful and suitable biochemical markers for evaluating and monitoring renal osteodystrophy.

Cross-linked C- and N-terminal telopeptides of type I collagen (CTx and NTx)

Cross-linked telopeptides of collagen type I, the resorption markers, are released into blood and excreted in the urine. Both CTx and Ntx can be easily measured in serum by immunoassays [9]. Cross-linked C-terminal telopeptide of the $\acute{a}_1$-chain of type I

collagen (s-CTx) is a sensitive marker useful in diagnosis of bone metabolism disturbances in renal osteodystrophy [23].

In one study new markers of bone metabolism were assessed in kidney transplant recipients including serum Cross Laps, TRAP and bAP. Serum CTx correlated with other markers of bone formation and resorption [24].

In another study s-CTx was measured in patients with chronic renal failure (CRF) before and after treatment. There was a significant positive correlation of s-CTx and serum creatinine, bAP and duration of disease. Patients with higher serum CTx level had significantly higher serum creatinine, phosphorus, bAP activity and longer duration of CRF. After 6 months of treatment a statistically significant decrease of s-CTx was observed. It was concluded that s-CTx in chronic renal failure patients can be a useful diagnostic marker of bone resorption changes after treatment of renal osteodystrophy [23].

N-telopeptides (NTx) are more specific than C-telopeptides because they contain both $á_1$ and $á_2$ chains, a feature common to all type I collagen, including non-bone tissue. NTx do not exhibit diurnal variation and can be measured in the serum by immunoassay as a stable end product of bone resorption.

Studies indicated that NTx may be a useful predictive marker to assess the effect of anti-resorptive therapies. The clinical utility of NTx in renal osteodystrophy is currently under investigation [25].

Other potential markers

Besides the biochemical serum markers of bone turnover other factors are involved in the process of bone remodeling including hormones, cytokines, growth factors, bone sialoprotein, $â_2$-microglobulin, osteoprotegerin and advanced glycation end products.

Parathyroid hormone (PTH) is a major regulator of bone turnover and skeletal cellular activity and it's measurement in serum or plasma has been widely used [2]. PTH in the serum occurs as intact hormone and many different PTH fragments. Coen *et al* showed higher predictive value of intact parathyroid hormone (iPTH) measurement in haemodialysis patients than in predialysis, in the non-invasive diagnosis of renal bone disease [26]. Moreover, they demonstrated that the PTH 1-84 to PTH 7-84 ratio is not a marker of low turnover osteodystrophy [27]. Qi *et al* showed that the measurement of serum iPTH levels alone were not able to distinguish adynamic or normal bone from hyperparathyroid bone disease [6,7]. The interpretation of PTH levels is complicated by skeletal resistance to PTH in chronic renal failure and its levels vary according to the type of dialysis, the degree of aluminium overload and probably other factors [7].

Results by Reichel *et al* showed that TRAP 5b, bAP and Oc correlate with intact PTH level and the whole PTH level. Their data suggest that both assays give similar information [28].

There is an evidence to suggest that various cytokines play a key role in the regulation of bone metabolism and might be altered in patients with chronic renal failure. The most important cytokines for bones are: interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-11 (IL-11), tumor necrosis factor- á (TNF-á) and transforming growth factor-â (TGF-â). Locally produced IL-1 or TNF-á induce proliferation and differentiation of osteoclast precursors.

High levels of IL-1 and high levels of IL-1 receptor antagonist have been reported in dialysis patients [29].

Other soluble cytokines involved in the development of osteoclasts are IL-6 and IL-11. The action of IL-6 depends on its circulating receptor. In the past years evidence has accumulated that supports the importance IL-6 in the pathophysiology of several diseases including renal osteodystrophy [30].

Another cytokine involved in bone remodeling is TNF-á found in high levels in uraemic patients. Both TNF-á and IL-1â stimulate bone resorption by their influence on the osteoclast's activity. They are also involved in secretion of IL-6 by osteoblasts and monocytes. Finally, both TNF-á and IL-1â inhibit bone formation [31]. It was shown that bone marrow space in patients with renal osteodystrophy accumulates IL-1á, IL-6, TNF-á and TGF-â. Also PTH increases IL-6 and TGF-â production in osteoblasts. This suggests that PTH can stimulate selective cytokine synthesis and that hyperparathyroidism may be a cause of cytokine accumulation in renal osteodystrophy [29,32].

Expression of IL-1, TNF-á, IL-6, IL-11 and their receptors is increased in end stage renal disease. This is suggested to play an important role in the activation of bone turnover in renal osteodystrophy [32].

Recent data indicated that the osteoprotegerin/osteoprotegerin-ligand (OPG/RANKL(OPGL)) cytokine complex which is produced by osteoblasts is involved in osteoclastogenesis [5]. OPG is a decoy receptor that inhibits osteoclast differentiation and bone resorption by blocking the interaction of nuclear factor–êB (RANK) with its ligand (RANKL). Intact PTH, 1,25 $(OH)_2D_3$, prostaglandin E2 and IL-11 act by stimulating RANKL production and inhibiting the synthesis of the OPG. Some data suggest that OPG, which accumulates in serum of uraemic patients might inhibit osteoclastogenesis induced by PTH [33]. The measurement of circulating OPG and iPTH levels might be important in renal osteodystrophy [4].

Haas *et al* demonstrated that OPG in combination with iPTH can be used as markers for non-invasive diagnosis of renal osteodystrophy in haemodialysis patients and that serum OPG levels might be used to estimate trabecular bone materialisation in these patients [34].

Results by Coen *et al* showed that the determination of serum OPG concentration could be used in the diagnosis of low turnover bone disease, at least associated with PTH levels of d"300 pg/ml [5].

Another study has demonstrated a 6-fold increase in serum OPG levels in dialysis patients compared with controls. Moreover, in dialysis patients with serum iPTH above 200 pg/ml OPG levels were higher than in patients with concentration of iPTH below 200 pg/ml [35].

The presence of $â_2$-microglobulin ($â_2m$, a polypeptide of amyloid that deposits in osteoarticular structures in haemodialysis patients, was first demonstrated by Argilès *et al* in 1989. Thereafter, results of several clinical trials have indicated that in glomerular kidney disease $â_2$microglobulin levels increase in the blood and decrease in the urine [36]. In tubular kidney disease urinary levels of $â_2$-microglobulin are raised and blood levels decreased. A high increase of serum $â_2m$ levels has been observed in anuric patients with end stage renal failure [10]. Serum $â_2m$ levels correlate with markers of bone formation, osteocalcin and bAP, and with a specific marker of bone resorption- serum free pyridinoline, but not with iPTH. Recently, it has been observed that patients with high bone turnover had greater serum $â_2m$ levels than patients with normal/low turnover.

Very recently Motomiya *et al* found that circulating α_2-macroglobulin-β_2-microglobulin complex may occur in patients with dialysis-related amyloidosis (DRA). It is suggested that α_2M-β_2m complex is an important pathological factor in DRA [36].

Advanced glycation end products (AGE) are formed in bone matrix protein by non-enzymatic reaction with sugars. AGE products also accumulate in the serum of uraemic patients. Patients with end stage renal disease displayed very high levels of AGE. Recent observations have indicated that AGEs enhanced osteoclast-induced bone resorption probably through the stimulation of IL-6 production [37].

Finally, bone sialoprotein (BSP) is also postulated as a new marker of bone turnover in several bone diseases [10]. Bone sialoprotein is a phosphorylated glycoprotein and accounts for approximately 5-10% of the noncollagenous proteins of bone. It might be a bone resorption indicator in diseases with increased bone turnover. Clinical trials have showed that BSP appears to be a sensitive marker of bone remodeling. Serum BSP levels were significantly higher in patients with bone metabolism disorders. Weak, but significant correlation was observed between serum BSP and the urinary PYD and DPD [38]. However, further investigations are needed to evaluate its sensitivity and accuracy for diagnosis of renal osteodystrophy.

The clinical utility of serum markers of bone turnover for evaluating and monitoring of renal osteodystrophy remains still on the investigation.

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