Original Article

Changes in serum osteoprotegerin and bone turnover markers in type 2 diabetic hemodialysis patients

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Abstract

Background: It is well known that type 2 diabetic patients easily have bone fracture regardless of normal-high bone mineral density. Otherwise, diabetic hemodialysis patients often have adynamic bone disease, but the precise mechanism underlying this disease remains unclear. Recently, osteoprotegerin（OPG）has been identified as a cytokine that inhibits osteoclast differentiation. The present study was undertaken to evaluate changes in serum OPG and other bone turnover markers in type 2 diabetic hemodialysis patients. We also evaluated the effect of type 2 diabetic change for mineral and bone disorder in hemodialysis patients.

Methods: The subjects were 18 diabetic patients and 34 non-diabetic patients who were receiving hemodialysis. All patients were male. Bone turnover markers, including serum OPG, intact osteocalcin（OC）, intact parathyroid hormone（iPTH）, tartrate-resistant acid phosphatase（TRAP）, and alkaline phosphatase activity（ALP）were measured, and bone volume was determined as the speed of sound（SOS）in the calcaneus by quantitative heel ultrasound.

Results: The SOS value in diabetic patients was significantly smaller than that in non-diabetic patients. The serum OPG level in diabetic patients was significantly higher than that in non-diabetic patients, whereas serum iPTH, OC（a marker of bone formation）and TRAP（a marker of bone resorption）were significantly lower in diabetic patients. There were no significant differences in serum ALP, adjusted calcium, phosphate, and aluminum levels between the two groups.

Conclusion: Type 2 diabetic hemodialysis patients have low bone volume regardless of high levels of OPG and low levels of serum iPTH, OC, and TRAP. These findings suggest type 2 diabetes is a major factor which influence mineral and bone disorder in hemodialysis patients and the effect for bone might be different from the effect under normal kidney function.

（Key words: type 2 diabetes mellitus, adynamic bone disease, bone turnover markers, osteoprotegerin, chronic kidney disease）

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Introduction

Chronic kidney disease-mineral and bone disorder (CKD-MBD) is a term used to describe disorders of bone and mineral metabolism accompanying CKD. Bone metabolic disorder in CKD-MBD at stage 5D is referred to as renal osteodystrophy, which is further divided into five types, including three with high metabolic turnover (mild bone disorder, mixed bone disorder, and osteitis fibrosa) and two with low metabolic turnover (adynamic bone disease and osteomalacia)[1]. These bone disorders can occur alone or in combination. In recent years, the incidence of high-turnover bone disorders has decreased and that of low-turnover bone disorders has increased in CKD patients due to improvements in treatment of secondary hyperparathyroidism, an increased incidence of diabetic nephropathy, and an increase in the age of hemodialysis patients[2]. Moreover, since aluminum-containing drugs are now generally contraindicated for CKD patients, adynamic bone disease has become a more frequent low-turnover bone disorder in CKD patients[3]. The incidence of adynamic bone disease in hemodialysis patients has been reported to be 10-50%[4].

The serum parathyroid hormone (PTH) concentration is low in many dialysis patients with adynamic bone disease, which suggests that excessive suppression of PTH secretion due to active vitamin D therapy or long-term hypercalcemia is involved in the disease. Differentiation of osteoblasts is promoted and apoptosis of osteoblasts is suppressed by activation of vitamin D receptors in osteoblasts[5, 6]. Therefore, impaired activation of vitamin D receptors in osteoblasts may be a cause of adynamic bone disease. Suppression of PTH secretion from parathyroid glands due to accumulation of magnesium is another possible cause. Adynamic bone disease is often seen in patients who are elderly or malnourished and in those receiving hemodialysis. The number of hemodialysis patients with diabetic nephropathy has increased in recent years and many diabetic hemodialysis patients have adynamic bone disease. However, it is well known that type 2 diabetic patients without renal failure often have bone fracture regardless of normal-high bone mineral density. Therefore, elucidation of the mechanism of bone metabolic disorders in diabetic hemodialysis patients is an important issue.

Osteoprotegerin (OPG) is a recently discovered cytokine that inhibits production of osteoclasts[7-9] and has possible roles in various bone metabolic disorders[10-12]. The function of OPG in type 2 diabetic hemodialysis patients is still unclear. In this study, we investigated the serum levels of OPG and bone turnover markers with the aim of determining the effect of type 2 diabetic change for mineral and bone disorder in hemodialysis patients.

Materials and Methods

1. Subjects

Fifty-two patients who were receiving maintenance hemodialysis at our institution or related hospitals were enrolled in the study in April 2005. The study was conducted according to the principles of the Declaration of Helsinki and with the permission of our institutional ethics committee. All patients provided written informed consent prior to participation. Three patients with liver or blood diseases were excluded. Females were excluded to eliminate the effect of postmenopausal osteoporosis.

The subjects included 52 males aged 40 to 87 years old (mean age: 63.8 ± 1.8 years old) who had been undergoing hemodialysis for 4 months to 25.6 years (mean duration: 6.5 ± 0.8 years). The patients were being treated twice or thrice weekly with standard bicarbonate dialysis using semisynthetic

membranes（dialysis filter surface area: 1.3-2.0 m^2）. Dry weight was targeted in each case to achieve a normotensive edema-free state. The subjects were divided by primary disease into a diabetic group（n=18）and a non-diabetic group（n=34）. All diabetic patients had type 2 diabetes, with 4 receiving insulin therapy, 2 taking sulfonylurea drugs, and 3 taking an α-glucosidase inhibitor.

2. Measurement methods

Blood samples were obtained at the beginning of the week before hemodialysis. Sera was stored at -20℃ until used for measurements of the serum levels of calcium（Ca）, phosphorus（IP）, intact parathyroid hormone（iPTH）, osteoprotegerin（OPG）and aluminum（Al）; and the levels of the bone formation markers alkaline phosphatase（ALP）and intact osteocalcin（OC）and the bone resorption marker tartrate-resistant acid phosphatase（TRAP）.

Measurements were made using a Biomedica enzyme-linked immunosorbent assay（ELISA）kit（Biomedica, Bensheim, Germany）for OPG; a Nichols IRMA kit（Nichols Institute, San Juan Capistrano, CA, USA）for iPTH; an Osteocalcin Test Kokusai-F kit（International Reagents, Tokyo, Japan）for OC; and a N-Assay ACP Nittobo kit（Nitto Boseki, Tokyo, Japan）for TRAP. The adjusted calcium level was calculated using Payne's formula.

Bone volume was measured at the same day after hemodialysis session by quantitative heel ultrasound using a CM-100 ultrasound densitometer（Furuno Electric Co., Ltd., Hyogo, Japan）[13]. The CM-100 measures the speed of sound（SOS）in the calcaneus with an intraassay CV of about 0.2%. The SOS value is significantly positively correlated with lumbar bone mineral density in the general population[13]. Generally, assessment of osteoporosis is performed using X-ray methods such as dual-energy x-ray absorptiometry（DEXA）. However, quantitative heel ultrasound is more convenient than X-ray and repeated measurements can safely be performed. Comparison of the two methods in hemodialysis patients showed that SOS obtained from quantitative heel ultrasound has a significant correlation with results obtained by DEXA, although the sensitivity and specificity of the SOS value are lower[14]. Thus, quantitative heel ultrasound appears to have the ability for monitoring bone volume in hemodialysis patients.

3. Statistical analysis

Values are presented as means ± standard error（SE）. StatView® was used for all statistical analyses. Differences between diabetic and non-diabetic groups were analyzed by Mann-Whitney U-test with $P<0.05$ considered significant.

Results

The characteristics of the patients in the diabetic and non-diabetic groups are shown in Table 1. There were no significant differences in age, BMI, duration of HD, and administered doses of calcium carbonate and vitamin D3 between the two groups. Ca, IP, Al and ALP also did not differ significantly between the groups, but the SOS value（reflecting bone volume）was significantly smaller in the diabetic group（$P<0.05$）.

The OPG level was significantly higher in the diabetic group than in the non-diabetic group（4.23 ± 0.60 vs. 2.93 ± 0.24 ng/ml, $p<0.05$）（Fig. 1）. In contrast, the diabetic group had significantly lower levels of the bone formation marker OC（11.52 ± 3.04 vs. 22.22 ± 3.68 ng/ml, $p<0.05$）（Fig. 2）, the bone

resorption marker TRAP (5.75 ± 0.61 vs. 8.00 ± 1.03 IU/l, $p<0.05$) (Fig. 3), and iPTH (104.0 ± 38.77 vs. 237.30 ± 36.14 pg/ml, $p<0.05$) (Fig. 3) compared to the non-diabetic group.

Table 1. Baseline characteristics of the patients

	All patients	DM patients	non-DM patients	P-value
n	52	18	34	
Age (years)	63.8 ± 1.8	64.5 ± 2.8	63.5 ± 2.3	0.81
BMI	21.5 ± 0.5	21.4 ± 0.5	21.5 ± 0.4	0.87
Duration of HD (years)	6.5 ± 0.8	6.4 ± 1.4	6.5 ± 0.9	0.91
Dose of calcium carbonate (g/day)	2.8 ± 0.3	2.5 ± 0.4	3.0 ± 0.2	0.34
Dose of vitamin D3 (μg/day)	0.16 ± 0.04	0.19 ± 0.05	0.14 ± 0.04	0.24
Adjusted Ca (mg/dl)	9.10 ± 0.26	9.12 ± 0.31	9.08 ± 0.21	0.68
P (mg/dl)	5.60 ± 0.52	5.62 ± 0.78	5.59 ± 0.38	0.72
Aluminum (μg/dl)	0.88 ± 0.11	0.88 ± 0.19	0.87 ± 0.08	0.65
ALP (mU/ml)	239.5 ± 24.5	226.4 ± 48.9	248.7 ± 13.5	0.23
SOS (m/s)	1477.2 ± 4.1	1464.4 ± 5.7	1484.0 ± 5.2	0.02

Values are given as the mean ± SE.

The significance of differences between diabetic (DM) and non-diabetic (non-DM) patients were analyzed by Mann-Whitney U-test.

Figure 1. Serum osteoprotegerin (OPG) concentrations in diabetic (DM) and non-diabetic (non-DM) hemodialysis patients. Data were analyzed by Mann-Whitney U-test.

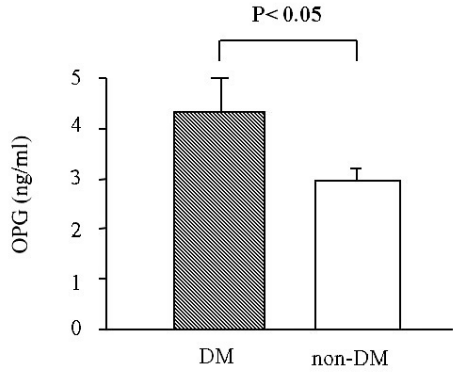


Figure 2. Serum osteocalcin（OC）concentrations in diabetic（DM）and non-diabetic（non-DM）hemodialysis patients. Data were analyzed by Mann-Whitney U-test.

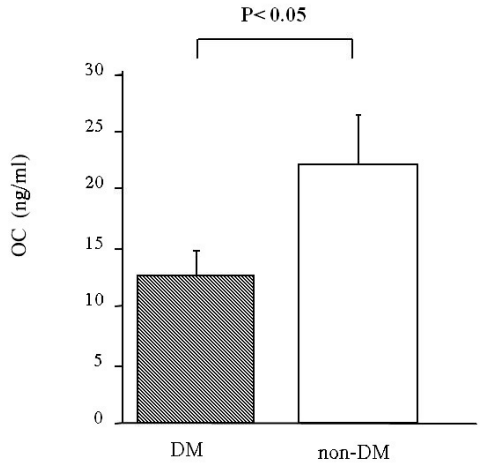
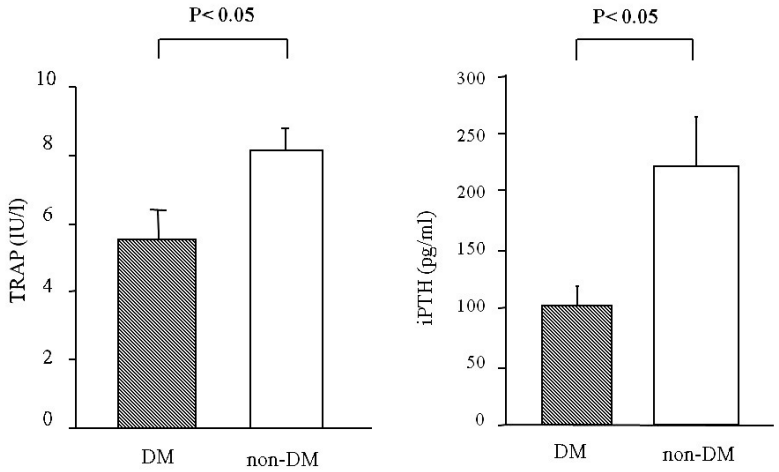


Figure 3. Serum intact PTH（iPTH）and tartrate-resistant acid phosphatase（TRAP）concentrations in diabetic（DM）and non-diabetic（non-DM）hemodialysis patients. Data were analyzed by Mann-Whitney U-test.



Discussion

This study showed an elevation in serum OPG and a decrease in OC, TRAP and iPTH in diabetic hemodialysis patients, and these changes suggest decreased bone turnover. However, type 2 diabetic hemodialysis patients had a lower bone volume than non-diabetic hemodialysis patients, suggesting the possibility of adynamic bone disease in diabetic hemodialysis patients. Thus, the disorder of glucose metabolism in diabetes has a major effect on bone metabolism, even in hemodialysis patients with renal

osteodystrophy. Since type 2 diabetic patients without renal failure have normal-high bone mineral density with same changes of bone turnover markers[15], bone metabolism in hemodialysis patients is different from that in diabetic patients with normal kidney function.

There are several possible mechanisms for the low bone turnover in diabetic hemodialysis patients. These include direct suppression of PTH secretion, since it has been shown *in vitro* that a high glucose concentration impairs the function of parathyroid cells and reduces secretion of PTH[16]. Clinically, Inaba et al.[17] showed that the serum PTH level in diabetic patients undergoing hemodialysis is significantly lower than that in non-diabetic hemodialysis patients, in agreement with our results. Another study showed that osteoblast function is impaired by apoptosis induced by advanced glycation end-products (AGE)[18]. It is also thought that low bone turnover is promoted by reduction of PTH secretion caused by nutritional deficiency in diabetic patients.

OPG is a 120 kDa secretory glycoprotein belonging to the TNF receptor superfamily. OPG inhibits differentiation of osteoclasts by acting on the decoy receptor activator of nuclear factors-kB ligand (RANKL), which is secreted by osteoblasts[6-8]. Osteoprotegerin is present in bone and can be detected in plasma. Hemodialysis patients show higher serum OPG levels than those in individuals with normal renal function[19], suggesting that OPG accumulates in blood due to a reduction in renal clearance accompanying kidney dysfunction. On the other hand, serum OPG is increased in diabetic patients with normal renal function compared to the level in non-diabetic patients[20]. If the serum OPG concentration is determined only by renal clearance, there should be no difference between OPG levels in diabetic and non-diabetic patients undergoing hemodialysis. However, the serum level of OPG in diabetic hemodialysis patients was clearly higher than that in non-diabetic hemodialysis patients in the present study, and was also higher than that in subjects with normal renal function.

It is unclear why serum OPG levels are high in diabetic hemodialysis patients. However, the results of a previous study indicated that interaction of core binding factor a1 (Cbfa1) and Smad proteins might mediate the effects of transforming growth factor-β (TGF-β) on transcription of OPG[21]. TGF-β is an important factor causing renal changes such as renal hypertrophy and extracellular matrix changes in diabetes[22]. These findings suggest that TGF-β, which promotes expression of OPG, might play a major role in the increase in serum OPG in diabetic hemodialysis patients.

An effect of OPG in diabetic osteopenia has been proposed in diabetic patients with normal kidney function and OPG might play a preventive role against the decrease of bone mineral density[15]. Moreover, it has been reported that the bone resorption marker ES/BS has a negative correlation with serum OPG in renal osteodystrophy[23], which suggests that serum OPG changes in accordance with the degree of bone resorption. Taken together, these findings suggest that the high serum level of OPG found in diabetic hemodialysis patients in the present study might reflect a preventive role of OPG against the low bone turnover seen in diabetic patients. However, the SOS value in type 2 diabetic hemodialysis patients was low in this study, and these findings suggest that their bone might have poor response to OPG.

OPG is also present in the walls of arteries and veins, and has been detected immunohistochemically in areas of intimal calcification and atherosclerotic plaque[24]. This indicates that OPG is a protective factor for endothelial cells, in which intimal injury readily occurs. In support of this possibility, atherosclerosis is predominant in OPG-deficient mice[25] and a high serum OPG level in patients with myocardial infarction is a predictor of a good prognosis[26]. Morena et al. found that a high serum OPG level in

hemodialysis patients predicts a poor prognosis[27], whereas a low serum OPG level has been shown to be a predictor of a poor prognosis in Japanese hemodialysis patients[28]. Thus, the protective effect of OPG on arteriosclerosis is uncertain and there may be significant racial or individual differences. Since OPG has effects on both bone and atherosclerosis, it appears to play an important role in the progression of cardiovascular diseases and bone metabolic disorders in diabetic patients.

Our study has the limitation of being observational in nature and was performed in a small number of subjects. Also, bone turnover markers and SOS value were only measured once. Due to the day-to-day variability of these markers, it is possible that the strength of the links among the bone turnover markers, SOS value and diabetes mellitus were under- or overestimated. However, within these limitations, we conclude that high levels of OPG and low levels of serum iPTH, OC, and TRAP may be involved in low bone volume in type 2 diabetic hemodialysis patients. These findings suggest type 2 diabetic is a major factor, which influence mineral and bone disorder in hemodialysis patients and the effect might be different from the effect under normal kidney function.

Disclosure

All the authors have declared no competing interest.

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２型糖尿病合併透析患者における血中破骨細胞形成抑制因子（osteoprotegerin）濃度および骨代謝マーカーの変化

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要　約

【目的】２型糖尿病では骨密度の低下を伴わない骨の脆弱性が問題となっている。一方，２型糖尿病合併の透析患者では無形成骨が多いと報告されている。近年破骨細胞形成抑制因子（osteoprotegerin: OPG）が発見され，破骨細胞の分化を抑制するとされている。今回，我々は，透析患者において血中 OPG 濃度および各種骨代謝マーカーを測定し，２型糖尿病が透析患者の骨ミネラル代謝異常にどのような影響を及ぼしているかについて検討した。

【方法】維持血液透析下の男性患者52名を２型糖尿病（DM）群18名と非糖尿病群34名に分け，透析開始前に採血し，各種骨代謝マーカー［副甲状腺ホルモン（iPTH），アルミニウム（Al），アルカリフォスファターゼ（ALP），オステオカルシン（OC），酒石酸抵抗性酸フォスファターゼ（TRAP）］と OPG を測定した。骨量指標として透析後に腫骨超音波法にて超音波伝播速度を測定し，the speed of sound（SOS）で示した。

【結果】DM 群の SOS は非糖尿病群に比して有意に低値だった。血中 OPG 濃度は DM 群では有意に高値であり，iPTH, OC, TRAP は逆に低値を示した。Ca, P, Al, ALP は有意差を認めなかった。

【考察】透析施行中の２型糖尿病患者では非糖尿病患者に比べ OPG 濃度が有意に高値を示しているにも関わらず骨量が低下していることから，透析施行期では糖尿病による骨ミネラル代謝に及ぼす影響は腎機能正常期とは異なることが示唆された。

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