

Bone mineral density may be related to atherosclerosis in hemodialysis patients

Ayumu Nakashima · Noriaki Yorioka · Chie Tanji
Yukiteru Asakimori · Rika Ago · Koji Usui
Kenichiro Shigemoto · Satoru Harada

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Abstract Biological interactions between the bone and the blood vessels are gradually being clarified. To investigate the relationship between bone mineral density and atherosclerosis in hemodialysis patients, we examined the bone mineral density and the intima-media thickness of the carotid artery in 83 dialysis patients with non-diabetic nephropathy (44 men and 39 women) aged from 23 to 83 years. The duration of hemodialysis ranged from 2 to 344 months. The bone mineral density of the radius was measured by dual-energy X-ray absorptiometry, and the ratio of this value to the standard value for the same age and gender was calculated (Z-score). As an index of atherosclerosis, the intima-media thickness of the carotid artery was measured by high resolution B-mode ultrasonography. Then the relationship between the Z-score and various factors was examined using Spearman's rank correlation analysis and multiple regression analysis. The Z-score showed a negative correlation with the duration of hemodialysis, the carotid intima-media thickness, and the levels of alkaline phosphatase, intact parathyroid hormone, and low-density lipoprotein cholesterol by Spearman's rank correlation analysis. In addition, the Z-score showed a positive correlation with the lipoprotein (a) level and a negative correlation with the duration of hemodialysis, intima-media thickness, intact parathyroid hormone, and low-density lipoprotein cholesterol by multiple regression analysis. These findings suggest that the decrease of bone mineral density in hemodialysis patients is correlated with secondary hyperparathyroidism and

hyperlipidemia, which are factors known to promote atherosclerosis, and thus bone density changes might be related to the progression of atherosclerosis, or vice versa.

Keywords Atherosclerosis · Bone mineral density · Hemodialysis · Intima-media thickness · Osteoporosis · Secondary hyperparathyroidism

Introduction

A significant decrease in bone mineral density (BMD) has been reported in hemodialysis patients when compared with the general population, and this decline of BMD becomes more marked as the duration of dialysis increases [1]. There is also a significant increase in atherosclerosis in dialysis patients when compared with the general population [2, 3].

Recently, it has been recognized that there is a close relationship between bone abnormalities and vasculopathy, with changes of the bone and the blood vessels being regulated by common factors. In postmenopausal women, it has been shown that a decrease of BMD is associated with the progression of atherosclerosis [4]. In addition, a correlation has been found between the BMD and the age-adjusted mortality rate or the death rate from heart failure [5]. However, the relationship between BMD and atherosclerosis has not yet been clarified in hemodialysis patients.

High-resolution B-mode ultrasonography has been widely used to non-invasively assess atherosclerosis of the carotid artery in epidemiologic and clinical studies performed in hemodialysis patients [2, 3], and it is thought that carotid artery changes mirror the progression of systemic atherosclerosis not only in the general population, but also in hemodialysis patients [6].

The present study was performed to investigate the relationship between BMD and atherosclerosis in hemodialysis patients. The radial BMD was measured as

A. Nakashima · R. Ago · K. Usui · K. Shigemoto · S. Harada
Department of Internal Medicine,
Harada Hospital, Hiroshima, Japan

N. Yorioka (✉) · C. Tanji · Y. Asakimori
Department of Molecular and Internal Medicine,
Graduate School of Biomedical Sciences, Hiroshima University,
1-2-3 Kasumi Minami-Ku, 734-8551 Hiroshima City, Japan
E-mail: nyorioka@hiroshima-u.ac.jp
Tel.: +81-82-2575196
Fax: +81-82-2557360

an index of osteoporosis and the intima-media thickness (IMT) of the carotid artery was measured as an index of atherosclerosis. Then the correlation between radial BMD and IMT was examined.

Materials and methods

Subjects

We enrolled 83 patients who were on hemodialysis at Harada Hospital (Hiroshima, Japan). We excluded patients with diabetic nephropathy because diabetes mellitus is a major independent risk factor for atherosclerosis. All of the patients were clinically stable and had no acute infection. We also excluded patients who had undergone parathyroidectomy or had a history of heavy aluminum exposure. All of the patients were taking calcium-containing binders and 12 patients who had secondary hyperparathyroidism were receiving treatment with active vitamin D metabolites. The underlying renal disease was chronic glomerulonephritis in 67 patients, nephrosclerosis in ten patients, polycystic kidney disease in two patients, and other conditions in four patients. The patients participated in this study after giving informed consent.

Measurement of radial BMD

We measured the BMD of the distal one-third of the radius by dual-energy X-ray absorptiometry (DXA) (DOS-600; Aloka, Tokyo, Japan). DXA measurements of the distal radius predominantly reflect cortical bone and the values obtained indicate the bone mass and not the volumetric bone density. In dialysis patients, the potential confounding effect of concurrent soft-tissue calcification on DXA values should be smaller at this site. In addition, the decrease in BMD is significantly greater for cortical bone than for cancellous bone in hemodialysis patients with secondary hyperparathyroidism [7]. The ratio of the actual BMD value measured by DXA to the standard BMD for the same age and gender was calculated to give the normalized value (BMD Z-score) [1]. We used the BMD Z-score to adjust BMD data, in order to compensate for the effect of menopause in postmenopausal female patients [4].

Carotid ultrasonography

Ultrasonography of the carotid artery was performed using a high resolution real-time scanner with a 7.5 MHz transducer (SSA-350; Toshiba, Tokyo, Japan). All subjects were examined by a single trained physician, who was blinded with regard to their clinical characteristics. Examination was performed in the supine position, and the carotid bifurcation and common carotid artery were scanned on both sides.

Scanning was done in three different longitudinal projections as well as the transverse projection. As defined by Wendelhag et al. [8], the IMT was measured at the site of greatest thickness (including plaque) and three measurements made at each site were averaged. Then the highest value among the six average IMT values (three from the left side and three from the right side) was used as the representative value for each patient [9]. To determine the intraobserver variability of IMT measurement, a total of 30 subjects were examined on two different occasions at an interval of 7–14 days. The coefficient of variation was only 2.0%.

Blood pressure

After enrollment in the study, four predialysis blood pressure readings were obtained from each patient. Then the mean arterial

pressure was calculated as the diastolic blood pressure plus one-third of the difference between the systolic and diastolic blood pressures, and the average of the four mean values was determined.

Biochemical parameters

Venous blood samples were collected after an overnight fast for measurement of the serum concentrations of alkaline phosphatase, intact parathyroid hormone (intact PTH), calcium, phosphate, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL cholesterol), and lipoprotein (a). Intact PTH was measured using the Nichols IRMA kit (Nichols Institute, San Juan Capistrano, Calif., USA). The adjusted calcium level was calculated using Payne's formula and the low-density lipoprotein cholesterol (LDL cholesterol) level was calculated using Friedewald's formula [10].

Statistical analysis

All results are shown as the mean \pm SD and statistical analysis was performed by the Mann-Whitney *U*-test. We used Pearson's correlation coefficient analysis to examine the relationship between the radial BMD Z-score and the IMT, as well as between the BMD Z-score and intact PTH. We also examined the relationship between the radial BMD Z-score and the following factors using Spearman's rank correlation analysis: duration of hemodialysis, body mass index, IMT value, systolic blood pressure, diastolic blood pressure, mean arterial pressure, and levels of alkaline phosphatase, intact PTH, adjusted calcium, serum phosphate, total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, and lipoprotein (a). Furthermore, multiple regression analysis was performed to evaluate possible predictors of the radial BMD Z-score. The variables tested were duration of hemodialysis, body mass index, IMT value, mean arterial pressure, and levels of PTH, adjusted calcium, serum phosphate, LDL cholesterol, and lipoprotein (a). Finally, multiple regression analysis was performed to evaluate possible predictors of the IMT value, which included age, gender (male), duration of hemodialysis, and intact PTH level. Analyses were performed with StatView 5 software (Abacus Concepts, Inc., Calif., USA) and $P < 0.05$ was considered to indicate a significant difference.

Results

Table 1 summarizes the clinical and biochemical parameters of the subjects. There were 44 men and 39 women aged from 23 to 83 years, with a mean age of 54.0 ± 13.0 years. The duration of hemodialysis ranged from 2 to 344 months, with a mean of 89.7 ± 80.7 months. A significant difference was observed between men and women with respect to radial BMD ($P < 0.0001$), total cholesterol level ($P = 0.0432$), and lipoprotein (a) level ($P = 0.0248$).

The radial BMD Z-score showed a negative correlation with the IMT value according to Pearson's correlation coefficient analysis ($R^2 = 0.113$, $P = 0.0019$, Fig. 1). The BMD Z-score was also negatively correlated with the intact PTH level (Pearson's $R^2 = 0.168$, $P = 0.0001$, Fig. 2). The factors assessed for a relationship with the radial BMD Z-score by Spearman's rank correlation analysis are shown in Table 2. There was a negative correlation with the duration of hemodialysis, the IMT value, the alkaline phosphatase level, the intact PTH level, and the LDL cholesterol level. Positive correlations

Table 1 Clinical and laboratory data of the subjects

	Normal values	Total (n=83)	Male (n=44)	Female (n=39)
Age (years)		54.0 ± 13.0	52.7 ± 12.8	55.4 ± 13.3
Duration of hemodialysis (months)		89.7 ± 80.7	87.4 ± 77.4	92.3 ± 85.2
Body mass index (kg/m ²)		21.1 ± 2.9	21.2 ± 2.2	21.1 ± 3.6
Radial bone mineral density (g/cm ²)		0.585 ± 0.135	0.645 ± 0.119	0.518 ± 0.120**
Radial BMD Z-score (%)		88.7 ± 16.9	86.9 ± 16.9	91.1 ± 16.7
IMT value (mm)		0.99 ± 0.54	1.04 ± 0.62	0.95 ± 0.44
Systolic blood pressure (mmHg)		158.4 ± 21.0	160.3 ± 20.6	156.3 ± 21.6
Diastolic blood pressure (mmHg)		85.5 ± 13.8	87.5 ± 12.2	83.3 ± 15.2
Mean arterial pressure (mmHg)		133.9 ± 17.4	136.0 ± 16.5	131.6 ± 18.3
Alkaline phosphatase (IU/l)	115–359	173.2 ± 86.7	163.2 ± 68.8	184.4 ± 103.0
Intact PTH (pg/ml)	10–65	228.1 ± 256.1	218.8 ± 203.2	238.6 ± 307.6
Adjusted calcium (mg/dl)	8.8–10.3	9.58 ± 0.97	9.55 ± 1.04	9.61 ± 0.91
Serum phosphate (mg/dl)	2.5–4.5	5.55 ± 1.24	5.58 ± 1.32	5.51 ± 1.16
Total cholesterol (mg/dl)	130–220	182.2 ± 41.0	173.2 ± 34.8	192.3 ± 45.3
Triglycerides (mg/dl)	50–150	137.0 ± 85.0	139.6 ± 92.5	134.1 ± 76.8
HDL-cholesterol (mg/dl)	35–75	49.4 ± 14.0	49.3 ± 15.0	49.4 ± 13.1
LDL-cholesterol (mg/dl)	< 120	105.8 ± 35.7	103.1 ± 30.3	108.9 ± 41.2
Lipoprotein (a) (mg/dl)	0–40	20.7 ± 14.9	18.5 ± 15.9	23.2 ± 13.5*

P* < 0.05, *P* < 0.001 (versus males)

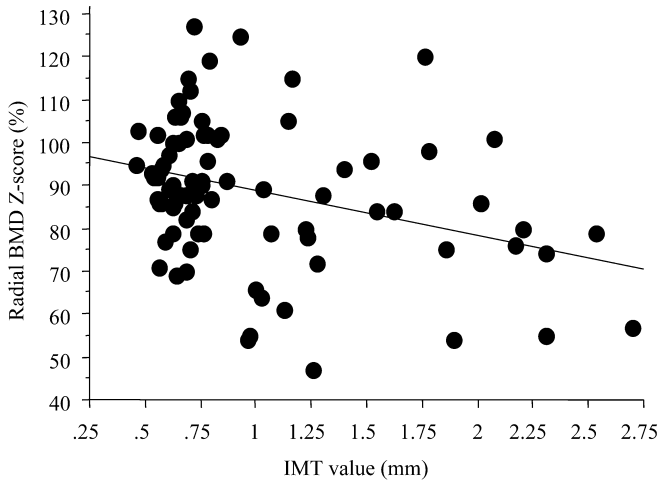


Fig. 1 Correlation between carotid IMT value and radial BMD Z-score. *n* = 83, *R*² = 0.113, *P* = 0.0019, *Y* = 99.12–10.46*X*

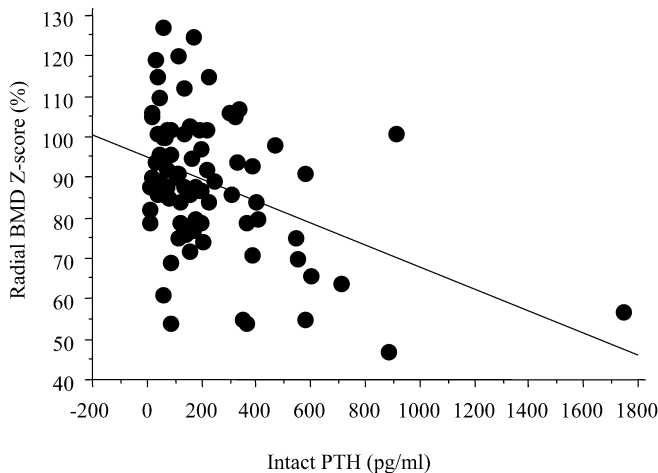


Fig. 2 Correlation between intact PTH level and radial BMD Z-score. *n* = 83, *R*² = 0.168, *P* = 0.0001, *Y* = 94.84–0.027*X*

Table 2 Spearman's rank correlation analysis of factors related to the radial BMD Z-score

	<i>ρ</i> -value	<i>P</i> -value
Duration of hemodialysis	-0.399	0.003
Body mass index	0.201	0.0693
IMT value	-0.283	0.0102
Systolic blood pressure	0.094	0.3984
Diastolic blood pressure	-0.25	0.8160
Mean arterial pressure	0.036	0.7497
Alkaline phosphatase	-0.378	0.0006
Intact PTH	-0.325	0.0033
Adjusted calcium	-0.0123	0.2604
Serum phosphate	0.142	0.2035
Total cholesterol	-0.094	0.5014
Triglycerides	0.116	0.2961
HDL-cholesterol	-0.101	0.3617
LDL-cholesterol	-0.311	0.047
Lipoprotein (a)	0.061	0.0615

were seen with the body mass index and the lipoprotein (a) level, but these did not reach significance.

The results of multiple regression analysis of predictors of the radial BMD Z-score are summarized in Table 3. The BMD Z-score showed a positive correlation with lipoprotein (a) level, while there were negative correlations with duration of hemodialysis, IMT value, intact PTH level, and LDL-cholesterol level.

In addition, the results of multiple regression analysis of predictors of the IMT are summarized in Table 4. The IMT value showed a positive correlation with age and intact PTH level, but there was no correlation with gender or with duration of hemodialysis.

Discussion

In the present study, the radial BMD Z-score showed a positive correlation with the lipoprotein (a) level, while there was a negative correlation with duration of

Table 3 Multiple regression analysis of predictors of the radial BMD Z-score

Independent variable	Standard regression coefficient	P-value
Duration of hemodialysis	-0.96	0.002
Body max index	0.097	0.2777
IMT value	-0.247	0.0063
Mean arterial pressure	0.020	0.8181
Intact PTH	-0.243	0.0099
Adjusted calcium	0.027	0.7731
Serum phosphate	0.088	0.3218
LDL-cholesterol	-0.202	0.0212
Lipoprotein (a)	0.254	0.0073
R ²	0.487	<0.0001

Table 4 Multiple regression analysis of predictors of the IMT value

Independent variable	Standard regression coefficient	P-value
Age	0.344	0.0014
Gender (male)	0.133	0.2016
Duration of hemodialysis	0.008	0.9386
Intact PTH	0.265	0.0150
R ²	0.178	0.0039

hemodialysis, IMT value, and levels of alkaline phosphatase, intact PTH, and LDL cholesterol.

It was previously reported that the BMD measured at the femoral neck and lumbar spine showed a positive correlation with the serum phosphate level, while showing a negative correlation with the duration of hemodialysis, PTH level, and alkaline phosphatase level [11]. Although there was no correlation with serum phosphate in the present study, our findings confirmed the other relationships. Furthermore, our study showed that the radial BMD Z-score was correlated with IMT value, LDL cholesterol level, and lipoprotein (a) level.

An increased lipoprotein (a) level is a major risk factor for cardiovascular disease. In a young and healthy population, Thorsen et al. [12] found a positive correlation between femoral neck BMD and lipoprotein (a) level. Lipoprotein (a) is thought to be involved in tissue maintenance or repair, but its relationship with BMD has not been confirmed in adults. In the present study, there was a positive correlation between radial BMD Z-score and lipoprotein (a) level, suggesting that lipoprotein (a) may play a role in strengthening the bones of hemodialysis patients.

Parhami et al. [13] reported that lipid peroxidation products have opposing effects on vascular cells and bone cells, showing that oxidized LDL caused an increase of arterial calcification, but decreased bone mineralization in vitro. Similarly, our study showed a negative correlation between the radial BMD Z-score and LDL cholesterol level. Accordingly, a high LDL cholesterol level might not only be a risk factor for the formation of atherosclerotic plaque, but also for a decrease in BMD.

In the present study, the intact PTH level was significantly correlated with the radial BMD Z-score and with the carotid IMT value. In hemodialysis patients, the IMT was previously reported to show a positive correlation with age, smoking, serum phosphorus, and PTH [2]. On the other hand, the decrease in radial BMD in hemodialysis patients has been ascribed to secondary hyperparathyroidism leading to bone demineralization [7]. Therefore, secondary hyperparathyroidism may be a shared risk factor for the progression of atherosclerosis and for the decrease of BMD. Because atherosclerosis increases in hemodialysis patients at the same time as they develop osteoporosis, it seems possible that the two conditions may be more closely related than has been realized so far.

In hemodialysis patients, vascular abnormalities are due to both atherosclerosis and Mönckeberg's medial calcific sclerosis. With regard to calcification in the media wall of arteries, Nishizawa et al. [14] have suggested a relationship between the calcification of arterial plaque and decrease of BMD in hemodialysis patients. In addition, there is a correlation between calcification of arterial plaque and non-HDL cholesterol level. Ectopic calcification of the arteries might lead to ischemia of various organs. In young hemodialysis patients, a strong association between coronary artery calcification and a high calcium×phosphate product was detected by a study using electron-beam computed tomography [15]. These studies indicate that the treatment of abnormal mineral metabolism is necessary to prevent vascular abnormalities.

There are several factors that seem to be common to the development of osteoporosis and vascular abnormalities. Both bone and atherosclerotic arteries contain osteopontin, bone morphogenetic protein, matrix Gla protein, type I collagen, and osteonectin. Asou et al. [16] prepared osteopontin knockout mice, and showed that osteopontin is required for angiogenesis, osteoclast recruitment, and subsequent resorption of bone. Min et al. [17] studied osteoprotegerin knockout mice, and found that osteoprotegerin reverses osteoporosis by inhibiting the activity of endosteal osteoclasts and also prevents arterial calcification. In addition, it has been shown that PTH, PTH-related peptide, and tumor growth factor- β can regulate the calcification of vascular cells in vitro.

In hemodialysis patients, vitamin K may be the most interesting factor. Vitamin K is also an essential cofactor for the site-specific carboxylation of matrix Gla protein and osteocalcin [18]. Luo et al. [19] created matrix Gla protein knockout mice. These mice showed the phenotype of warfarin-induced bone abnormalities. In addition, all of the animals developed severe calcification of the major arteries and died within 6–8 weeks after birth due to rupture of the thoracic or abdominal aorta. This study clearly showed the vital importance of matrix Gla protein for preventing vascular calcification. The fact that matrix Gla protein expression is strongly induced in both atherosclerosis and Mönckeberg's medial calcific

sclerosis suggests that contact between tissues and calcium salt crystals is a common trigger in both processes. Vitamin K₂ increases bone formation and decreases bone resorption, and the vitamin K level is significantly reduced in dialysis patients [20]. Moreover, it has been reported that the fracture rate increases as the vitamin K level declines in hemodialysis patients [21]. Vitamin K₁ is found in green-yellow vegetables, while vitamin K₂ is found in fermented foods such as cheese. In hemodialysis patients, it is thought that intake of vitamin K is insufficient because of restrictions on the dietary intake of potassium and phosphate. Therefore, there is a possibility that arterial calcification and bone loss (a decrease in BMD) may show simultaneous progression in dialysis patients, and these observations suggest that osteoporosis and atherosclerosis may share some common mechanisms.

In conclusion, the present study revealed that the decrease of BMD in hemodialysis patients is correlated with secondary hyperparathyroidism and hyperlipidemia, which have been proven to promote atherosclerosis, and thus might be related to atherosclerosis.

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