Showa Univ J Med Sci **27**(2), 103～110, June 2015

Original

The Relationship Between Serum Homocysteine Levels and Vertebral Fractures

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Abstract: Serum homocysteine and pentosidine levels have attracted attention as associated markers of bone quality, which affects bone strength. We examined the relationship of serum homocysteine levels with existing vertebral fractures and renal function. We evaluated 279 of 960 osteoporosis outpatients (12 men, 267 women; mean age, 72 years) whose serum homocysteine levels had been measured in our department. Using a glomerular filtration rate (GFR)-based chronic renal failure severity classification system, we divided patients into three groups: G1/G2, G3a/G3b/G4, and G5. We further divided the patients in the G1/G2 and G3a/G3b/G4 groups into two subgroups on the basis of the presence of fractures. Vertebral fractures were significantly more frequent when serum homocysteine levels were high in the G1/G2 group ($P = 0.002$). Serum homocysteine levels were lower in patients in the G1/G2 group than the G3a/G3b/G4 group despite the presence or absence of vertebral fractures ($P < 0.001$). Significant differences in serum homocysteine levels were also seen between patients with and without vertebral fractures in both the G1/G2 and G3a/G3b/G4 groups ($P = 0.02$). There were also significant correlations between GFR and serum homocysteine levels in both the G1/G2 and G3a/G3b/G4 groups (correlation coefficients, −0.43 and −0.65, respectively; $P < 0.001$). A negative correlation was observed between serum homocysteine levels and GFR in the G1/G2 and G3a/G3b/G4 groups, and we were able to reaffirm that serum homocysteine levels are affected by renal function. In the G1/G2 group, the prevalence of vertebral fractures was significantly higher in patients with high serum homocysteine levels. Even if renal function was poor, serum homocysteine levels were significantly higher in patients with vertebral fractures. Thus, serum homocysteine is a valid marker of bone quality.

Key words: osteoporosis, serum homocysteine, vertebral fractures, bone quality, glomerular filtration rate

Introduction

Bone strength is a combination of bone mineral density and bone quality, with the former contributing to 70% of bone strength and the latter 30%[1]. Bone quality is conventionally assessed by bone turnover, microstructures, microfractures, and calcification. However, serum

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homocysteine and pentosidine levels are beginning to attract attention as associated markers of bone quality[2], because there is a correlation between serum pentosidine levels and vertebral fractures in women with type 2 diabetes mellitus[3] ; moreover, high serum homocysteine levels show a significant association with osteoporotic fractures[4, 5].

On the other hand, serum homocysteine and pentosidine levels are useful for the clinical assessment of atherosclerosis and renal dysfunction. Homocysteine has two fates. If there is a deficiency of methionine, homocysteine may be remethylated to methionine. If methionine stores are adequate, homocysteine may enter the transsulfuration pathway, where it is converted to cysteine. Because methionine is an essential amino acid, cysteine synthesis can be sustained only if the dietary intake of methionine is adequate[6]. In cases of hyperhomocysteinemia, there is an increased risk of atherosclerosis, which leads to a greater incidence of heart attacks caused by coronary artery disease and cerebral haemorrhage[7]. Because vascular calcification affects renal function, and comorbid hyperhomocysteinemia is common in dialysis patients, it is thought that homocysteine levels are implicated in renal function. Renal dysfunction, including hemodialysis, appears to be one cause of osteoporosis. The causes of hyperhomocysteinemia in patients with renal dysfunction are thought to be : (1) decreased hepatic uptake of homocysteine ; (2) decreased utilization of vitamin B_6, vitamin B_{12}, and folic acid, which are co-factors of the metabolizing enzymes ; and (3) the inhibition of metabolic factors leading to high levels of homocysteine being present in the blood[8].

In the present study, a widely-used classification system defining the severity of renal dysfunction (G1-G5), which is based on the glomerular filtration rate (GFR)[9], was used to group patients according to renal function. Then, we investigated whether homocysteine levels in the blood were related to existing vertebral fractures and examined its usefulness as a marker of bone metabolism.

Patients and methods

Patients

Out of a total of 960 osteoporosis outpatients whose serum homocysteine levels were measured in our department, we evaluated 279 patients (12 men and 267 women ; mean age, 72 years) from a mixed treatment group, comprising 28 patients receiving vitamin D_3, 15 receiving etidronate, 58 receiving alendronate, 59 receiving risedronate, 7 receiving minodronate, 20 receiving a selective estrogen receptor modulator (SERM), 5 receiving parathyroid hormone (PTH), and 87 not receiving any treatment.

Methods

In a GFR-based classification system for the severity of chronic renal failure, G1 represents normal or high GFR levels, G2 represents normal or mildly decreased levels, G3a represents mildly to moderately decreased levels, G3b represents moderately to severely decreased levels, G4 represents severely decreased levels, and G5 represents end-stage renal failure[9]. In the present study, patients were classified into two groups, G1/G2 and G3a/G3b/G4, while the patients in the

G5 group were excluded because of renal dysfunction (five patients; one man, four women; mean age, 75.8 years). We further divided the G1/G2 group (203 patients; 10 men, 193 women; mean age, 69.7 years) and the G3a/G3b/G4 group (71 patients; one man, 70 women; mean age, 79.4 years) into subgroups based on serum homocysteine levels and the prevalence of vertebral fractures. We measured serum homocysteine levels and designated ≦ 11.7 nmol/ml as 'normal' and > 11.7 nmol/ml as 'high'. In addition, we examined the correlation between serum homocysteine levels and GFR in the G1/G2 and G3a/G3b/G4 groups. We also examined whether there was a difference in the frequency of fractures in the G1/G2 and G3a/G3b/G4 groups within the normal and high serum homocysteine level groups.

Using serum creatinine (Cr) levels, GFR was calculated using the formulae:

$$\text{eGFR (ml/min/1.73 m}^2\text{)} = 194 \times \text{Cr (}\mu\text{M)}^{-1.094} \times \text{age (years)}^{-0.287} \text{ for men;}$$

$$\text{eGFR (ml/min/1.73 m}^2\text{)} = 194 \times \text{Cr}^{-1.094} \times \text{age}^{-0.287} \times 0.739 \text{ for women}^{9)}.$$

Statistical analysis

We analyzed differences in the frequency of existing vertebral fractures in the normal and high serum homocysteine level groups using the chi-squared test. The correlation between serum homocysteine levels and GFR in the G1/G2 and G3a/G3b/G4 groups was examined using the Spearman rank correlation coefficient. The Mann-Whitney *U* test was used to analyze the relationship between vertebral fractures and serum homocysteine levels in the G1/G2 and G3a/G3b/G4 groups. Serum homocysteine levels are presented as mean levels ± standard deviations, and the two-tailed significance level was set at 5%. Stat Flex ver. 6 statistical software (Artech Co., Ltd., Osaka, Japan) was used for all statistical analyses.

Ethical considerations

The Ethical Committee of Showa University School of Medicine (Tokyo, Japan) approved the protocols used in this study and the personal information of the study participants was protected (permit no. 1287).

Results

Frequency of existing vertebral fractures, based on serum homocysteine levels and GFR classification

In the normal serum homocysteine level subgroup of the G1/G2 group, there were 57 patients (33.3%) with vertebral fractures and 114 patients (66.7%) without vertebral fractures. In the high serum homocysteine level subgroup of the G1/G2 group, there were 20 patients (62.5%) with vertebral fractures and 12 patients (37.5%) without ($P = 0.002$; Fig. 1a). In the G1/G2 group, the number of vertebral fractures increased as the level of homocysteine increased.

In the normal serum homocysteine subgroup of the G3a/G3b/G4 group, there were 17 patients (44.7%) with vertebral fractures and 21 patients (55.3%) without. In the high serum homocysteine level subgroup of the G3a/G3b/G4 group, there were 21 patients (63.6%) with vertebral fractures and 12 patients (36.4%) without, but there were no significant differences between

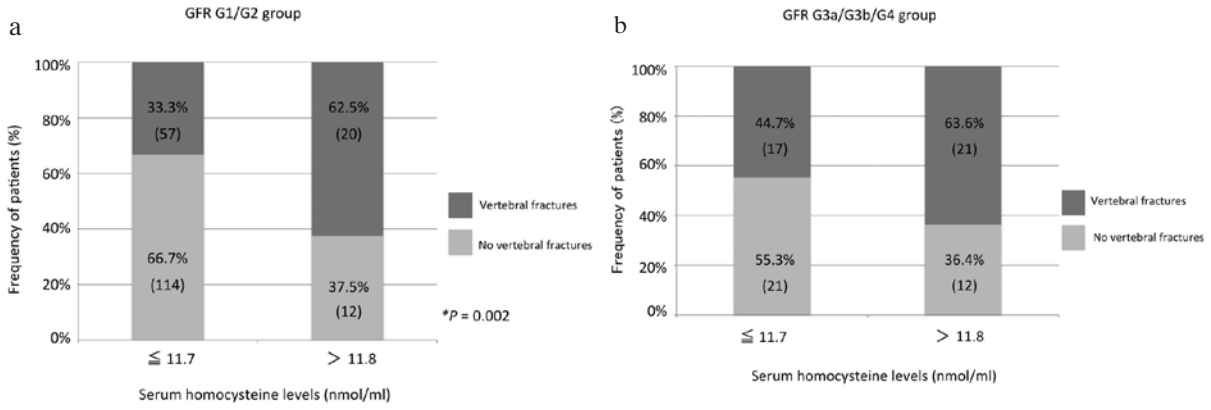

Fig. 1. Percentage (%) of patients with or without vertebral fractures in the normal and high serum homocysteine subgroups of the glomerular filtration rate (GFR) -based classification groups, (a) G1/G2, and (b) G3a/G3b/G4. $^{*}P = 0.002$, significant difference between subgroups.

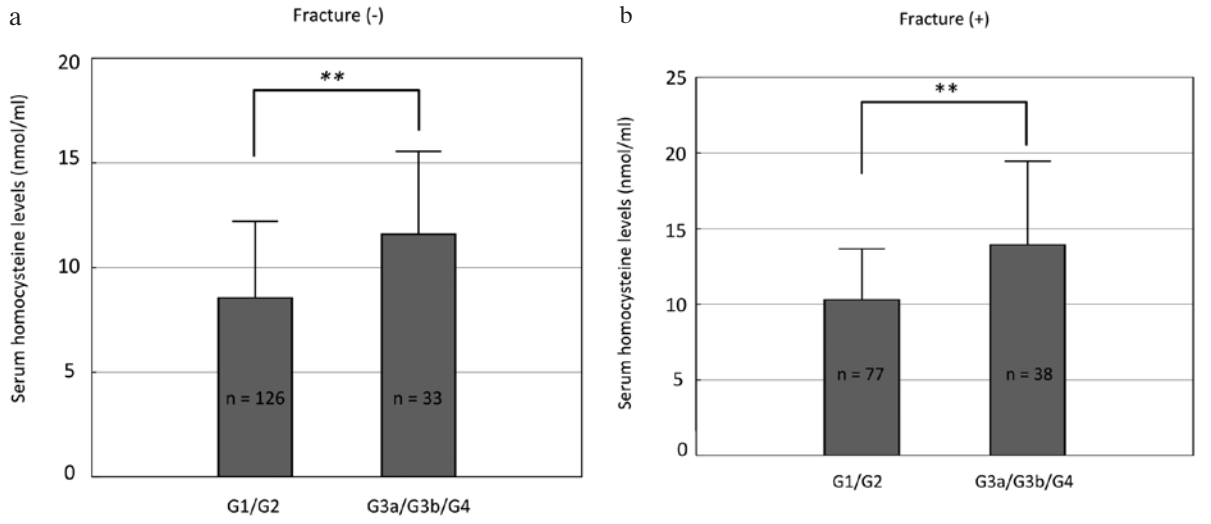

Fig. 2. Mean serum homocysteine levels (nmol/ml) in patients in the glomerular filtration rate (GFR)-based classification groups, G1/G2 and G3a/G3b/G4, (a) without, and (b) with vertebral fractures. $^{**}P < 0.001$, significant difference between groups.

groups ($P = 0.11$; Fig. 1b). As homocysteine was influenced by renal function in the G3a/G3b/G4 group, it was unclear if homocysteine levels had an effect on fractures.

Relationship between serum homocysteine levels and GFR classification, based on existing vertebral fractures

Among the patients without existing vertebral fractures, the mean serum homocysteine level was 8.6 ± 3.7 nmol/ml in the 126 patients in the G1/G2 group and 11.6 ± 4.0 nmol/ml in the 33 patients in the G3a/G3b/G4 group ($P < 0.001$; Fig. 2a).

Among the patients with existing vertebral fractures, the mean serum homocysteine level was

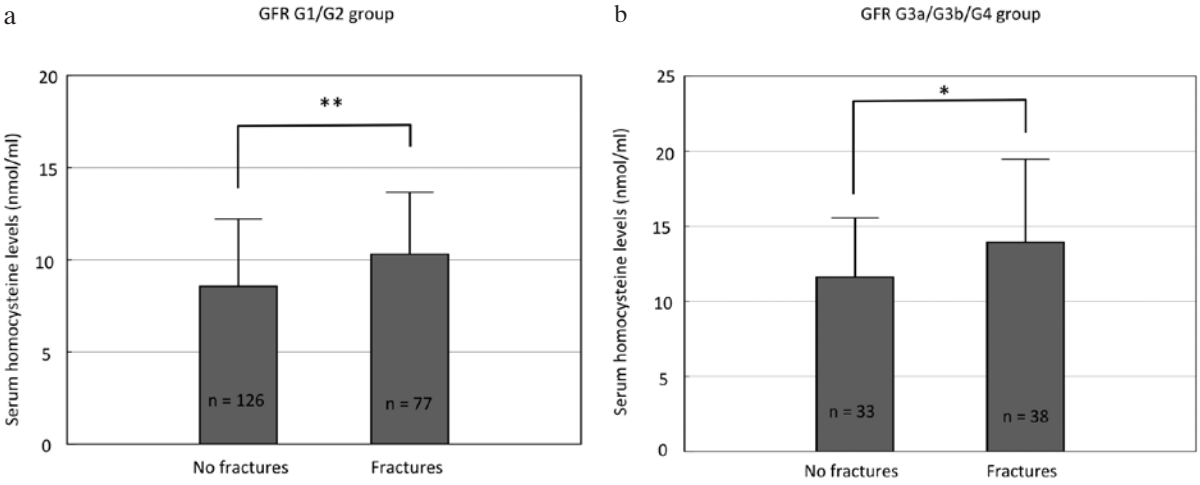

Fig. 3. Mean serum homocysteine levels (nmol/ml) in patients with and without vertebral fractures in the glomerular filtration rate (GFR)-based classification groups, (a) G1/G2, and (b) G3a/G3b/G4. $^{*}P < 0.02$; $^{**}P < 0.001$, significant difference between groups.

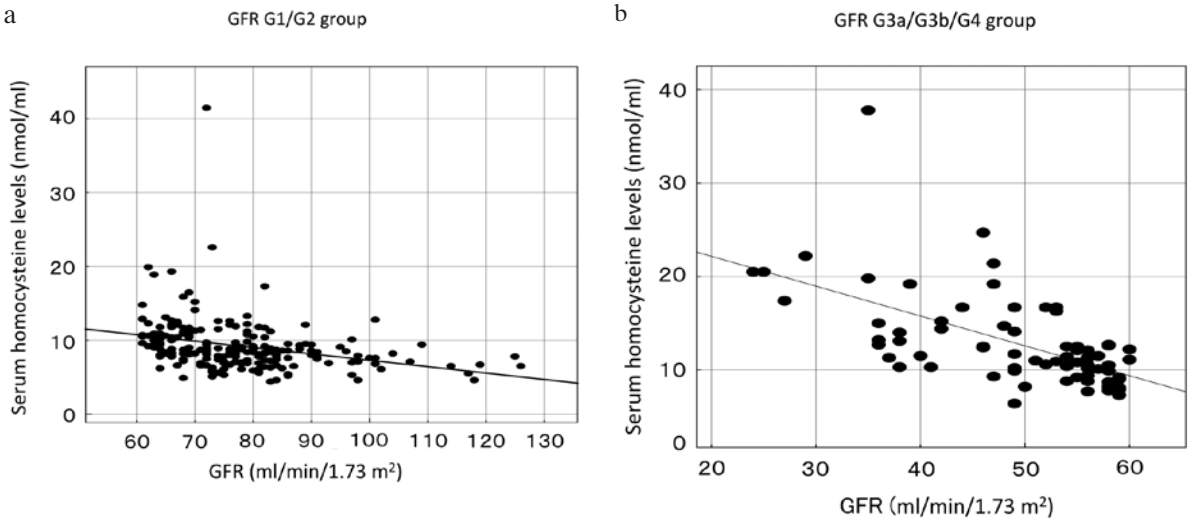

Fig. 4. Correlation between serum homocysteine levels (nmol/ml) and glomerular filtration rate (GFR; ml/min/1.73 m^2) in patients in the GFR-based classification group, (a) G1/G2 (Spearman rank correlation coefficient $=-0.43$, $P < 0.001$), and (b) G3a/G3b/G4 (Spearman rank correlation coefficient $=-0.65$, $P < 0.001$).

10.3 ± 3.4 nmol/ml in the 77 patients in the G1/G2 group and 13.9 ± 5.5 nmol/ml in the 38 patients in the G3a/G3b/G4 group ($P < 0.001$; Fig. 2b).

Relationship between serum homocysteine levels and vertebral fractures, based on GFR classification

Among the patients in the G1/G2 group, the mean serum homocysteine level was 10.3 ± 3.4 nmol/ml, in the 77 patients with vertebral fractures, whereas it was 8.6 ± 3.7 nmol/ml in the

126 patients without vertebral fractures ($P < 0.001$; Fig. 3a).

Among the patients in the G3a/G3b/G4 group, the mean serum homocysteine level was 13.9 ± 5.5 nmol/ml in the 38 patients with vertebral fractures, whereas it was 11.6 ± 4.0 nmol/ml in the 33 patients without vertebral fractures ($P < 0.02$; Fig. 3b).

Correlation between serum homocysteine levels and GFR classification groups

In both the G1/G2 and G3a/G3b/G4 groups, there was a significant correlation between GFR and serum homocysteine levels (Spearman rank correlation coefficients, −0.43 and −0.65, respectively; $P < 0.001$; Figs. 4a and 4b).

Discussion

The serum homocysteine level is considered a useful marker of bone quality and renal function. Our results showed that in the G1/G2 group, serum homocysteine levels showed significant differences between patients with and without vertebral fractures. From these results, it is possible to assume that serum homocysteine can be used as an index of existing vertebral fractures when renal function is normal or mildly decreased. In the G3a/G3b/G4 group, there was no difference in the number of patients with or without an existing vertebral fracture; however, serum homocysteine levels increased with vertebral fractures irrespective of the GFR-based renal dysfunction classification grade. Yokokawa *et al*[10] reported normal serum homocysteine levels in patients with tibial diaphyseal fractures who were taking bisphosphonates. Thus, we believe that serum homocysteine levels do not increase because of fractures; instead, we hypothesize that fractures may occur more frequently in patients with relatively higher homocysteine levels.

Our results indicated a correlation between serum homocysteine and GFR in both the G1/G2 and G3a/G3b/G4 groups, which confirmed that homocysteine is affected by renal function. Homocysteine is an amino acid produced in the metabolic pathway of the essential amino acid, methionine, and is resynthesized into methionine through the action of folic acid and vitamin B_{12}[6, 11]. As a result, oxidative stress is increased due to high levels of homocysteine. In addition, vitamin B_6, B_{12}, and folic acid insufficiencies cause abnormalities in bone collagen cross-linking[12] and bone becomes fragile despite the same bone mineral density. Low bone mineral density is an independent risk factor for fractures[4, 5, 12–15]. In addition, serum homocysteine levels are not correlated with the levels of bone metabolism markers such as bone alkaline phosphatase, deoxypyridinoline, and bone tartrate-resistant acid phosphatase-5b. Thus, bone metabolism markers are also considered independent factors for predicting fracture risk[4]. In contrast, methionine is mainly metabolized by the kidneys; therefore, when renal function decreases, normal metabolism is disturbed, causing homocysteine levels to increase. When hyperhomocysteinemia continues, it creates a negative cycle of further renal dysfunction associated with renal artery atherosclerosis[7, 8].

Hormones involved in bone resorption include vitamin D which is hydroxylated in the liver and proximal renal tubules and converted to the active form of vitamin D_3. The first hydroxylation occurs at the 25-position, and is catalyzed by a specific hydroxylase in the liver. The prod-

uct of the reaction, 25-hydroxycholecalciferol (25-OH-D_3), is the predominant form of vitamin D in the plasma and the major storage form of the vitamin. 25-OH-D_3 is further hydroxylated at the 1 position by 25-OH-D_3 1-hydroxylase found primarily in the kidney, resulting in the formation of 1,25-dihydroxycholecalciferol[16]. The active form of vitamin D_3 promotes the active absorption of calcium and phosphorus in the intestinal tract. Therefore, decreased renal function leads to impaired hydroxylation of vitamin D, causing osteoporosis and making vertebral fractures more likely to occur.

In cases with high bone turnover and increased bone resorption, the use of bisphosphonates and SERM is recommended for the inhibition of bone turnover. In contrast, there are cases in which vertebral fractures occur easily even when bone mineral density is high, such as in diabetes; therefore, high bone mineral density is not necessarily a good indicator of bone quality. When bone mineral density is low and associated markers of bone quality are high, increasing bone mineral density should be given priority and administration of bisphosphonates and PTH is recommended. However, bisphosphonates cannot easily improve the levels of markers associated with bone quality[1]. Therefore, clinically, SERM, PTH, and eldecalcitol are used to improve bone quality. PTH is considered effective in either case, but it can only be used for a maximum of 2 weeks because side effects need to be considered. SERM also increases bone mineral density, but the rate of increase is small compared with bisphosphonates[17]. Selection of a drug or drug combination varies according to the number of existing vertebral fractures, bone mineral density, bone metabolism markers, the state of bone quality, complications such as diabetes or chronic respiratory disease, the presence or absence of steroids or dental treatment, duration after menopause, and the state of renal function; therefore, a personalized medication regimen is necessary for each individual case.

There were some limitations in this study. First, there was a number of patients who received different osteoporosis medications, while some did not receive any drugs. Second, the SERM and PTH preparations were used to improve bone quality; therefore, these drugs may have affected serum homocysteine levels. We plan to continue our examination of whether serum homocysteine levels can be improved through osteoporosis treatment.

In conclusion, among patients in the G1/G2 group of the chronic renal failure GRF-based classification (normal to mild renal dysfunction), there was a significantly higher prevalence of vertebral fractures when serum homocysteine levels were high. Even in patients with poor renal function, serum homocysteine levels were significantly higher when there were vertebral fractures. Thus, our results suggest that the serum homocysteine level can be considered a valid marker of bone quality.

Acknowledgment

The authors thank Kenichi Matsui (Showa University Research Promotion Office) for assistance with the statistical analysis terms.

Conflict of interest disclosure

The authors have declared no conflict of interest.

References

1) NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy. Osteoporosis prevention, diagnosis, and therapy. *JAMA*. 2001;**285**:785-795.
2) Saito M, Marumo K, Soshi S, *et al*. Raloxifene ameliorates detrimental enzymatic and nonenzymatic collagen cross-links and bone strength in rabbits with hyperhomocysteinemia. *Osteoporos Int.* 2010;**21**:655-666.
3) Yamamoto M, Yamaguchi T, Yamauchi M, *et al*. Serum pentosidine levels are positively associated with the presence of vertebral fractures in postmenopausal women with type 2 diabetes. *J Clin Endocrinol Metab*. 2008;**93**:1013-1019.
4) McLean RR, Jacques PF, Selhub J, *et al*. Homocysteine as a predictive factor for hip fracture in older persons. *N Engl J Med*. 2004;**350**:2042-2049.
5) van Meurs JB, Dhonukshe-Rutten RA, Pluijm SM, *et al*. Homocysteine levels and the risk of osteoporotic fracture. *N Engl J Med*. 2004;**350**:2033-2041.
6) Harvey RA, Ferrier DR. Amino acid degradation and synthesis. In *Biochemistry*. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2011. pp261-275. (Lippincott's illustrated reviews).
7) Perry IJ, Refsum H, Morris RW, *et al*. Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. *Lancet.* 1995;**346**:1395-1398.
8) Arnadottir M, Hultberg B, Nilsson-Ehle P, *et al*. The effect of reduced glomerular filtration rate on plasma total homocysteine concentration. *Scand J Clin Lab Invest.* 1996;**56**:41-46.
9) Japanese Society of Nephrology. Clinical practice guidebook for diagnosis and treatment of chronic kidney disease 2012 Tokyo: Tokyo Igakusya; 2012. (in Japanese).
10) Yokokawa A, Kuroda K, Nishimura T, *et al*. The insufficiency fracture of femoral shafts in 5 patients on long-term bisphosphonate therapy for osteoporosis. *Osteoporos Jpn.* 2011;**19**:229-233. (in Japanese).
11) Finkelstein JD, Martin JJ. Methionine metabolism in mammals. Adaptation to methionine excess. *J Biol Chem.* 1986;**261**:1582-1587.
12) Saito M, Fujii K, Marumo K. Degree of mineralization-related collagen crosslinking in the femoral neck cancellous bone in cases of hip fracture and controls. *Calcif Tissue Int.* 2006;**79**:160-168.
13) Yazdanpanah N, Zillikens MC, Rivadeneira F, *et al*. Effect of dietary B vitamins on BMD and risk of fracture in elderly men and women: the Rotterdam study. *Bone.* 2007;**41**:987-994.
14) Shiraki M, Urano T, Kuroda T, *et al*. The synergistic effect of bone mineral density and methylenetetrahydrofolate reductase (MTHFR) polymorphism (C677T) on fractures. *J Bone Miner Metab*. 2008;**26**:595-602.
15) Saito M, Mori S, Mashiba T, *et al*. Collagen maturity, glycation induced-pentosidine, and mineralization are increased following 3-year treatment with incadronate in dogs. *Osteoporos Int.* 2008;**19**:1343-1354.
16) Harvey RA, Ferrier DR. *Vitamin D.* In *Biochemistry*. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2011. pp386-389. (Lippincott's illustrated reviews).
17) Nagai T, Sakamoto K, Matsunaga A, *et al*. Effects of 3 years of treatment with a selective estrogen receptor modulator for postmenopausal osteoporosis on markers of bone turnover and bone mineral density. *Showa Univ J Med Sci.* 2012;**24**:301-308.

[Received January 6 2015: Accepted April 10, 2015]