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Original article

Effect of denosumab on renal function in women with osteoporosis evaluated using cystatin C

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ABSTRACT

Objectives: To investigate renal function during denosumab therapy using the estimated glomerular filtration rate based on cystatin C (eGFRcys) which is more accurate than creatinine (eGFRcr) for renal function.

Methods: Bone mineral densities (BMDs) of lumbar spine and hip regions, eGFRcys, eGFRcr, creatinine clearance (Ccr), and serum total homocysteine (S-Hcy) were measured during 2-year denosumab therapy in 53 women with osteoporosis naïve to anti-osteoporosis drugs (new group) and 64 women who were switched from long-term bisphosphonate treatment to denosumab therapy (switch group).

Results: There were no significant differences in age, eGFRcr, Ccr, eGFRcys, and S-Hcy levels at baseline between the groups. BMDs in the lumbar spine, femoral neck, and total hip increased significantly after 2-year denosumab therapy in both groups. eGFRcr decreased in the switch group, and Ccr decreased in both groups; however, eGFRcys and S-Hcy levels did not change significantly in either group. To investigate the causal factors associated with the decrease in eGFRcr and Ccr, multiple regression analysis was performed in all patients. Denosumab initiation within 3 months after fracture and eGFRcr or Ccr at baseline were independent factors for the decrease in eGFRcr or Ccr during the 2-year denosumab therapy. Decline in creatinine-based renal function could be reflected by increased muscle mass during the ongoing recovery from fracture.

Conclusions: Renal function was preserved in all patients, including those in the switch group during denosumab therapy. Creatinine-based renal function should be cautiously interpreted during denosumab therapy in patients with recent fractures.

1. Introduction

Currently, oral bisphosphonates are commonly employed for the treatment of osteoporosis in Japan [1]. Renal function before and during treatment with bisphosphonates should be carefully monitored because bisphosphonates are exclusively excreted via the kidneys and, therefore, may adversely affect kidney function, especially in long-term treatment with bisphosphonates [2]. All bisphosphonate treatments should be administered cautiously to patients with a creatinine clearance (Ccr) of < 30 mL/min [3]. Denosumab, a monoclonal antibody to the receptor activator of nuclear factor κ-B ligand, suppresses osteoclastic function and differentiation from immature to mature osteoclasts, resulting in strong anti-resorption efficacy for osteoporosis [4–6]. It is administered once every 6 months for more long-term patient adherence than that with oral bisphosphonates [7,8]. Denosumab has lower adverse effects on the kidneys than bisphosphonates as denosumab disappears from the bloodstream after being degraded in the spleen [9]. In clinical practice, the estimated glomerular filtration rate based on creatinine (eGFRcr) occasionally decreases during osteoporosis drug therapy, including denosumab therapy. Renal function is commonly evaluated by eGFRcr; however, the estimated glomerular filtration rate based on serum cystatin C (eGFRcys) is more accurate than eGFRcr because serum creatinine levels are influenced by muscle mass and diet [10–12]. Thus far, in addition to a post-hoc analysis of controlled randomized trials of denosumab

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and the Fracture Reduction Evaluation of Denosumab in Osteoporosis Every 6 Months (FREEDOM) study [13], only 1 study has demonstrated longitudinal changes in renal function during denosumab therapy [14]. Bisphosphonates are often substituted with denosumab when bone mineral density (BMD) decreases or new osteoporotic fractures occur during long-term bisphosphonate therapy. Therefore, it is important to examine whether renal function can be preserved in cases in which bisphosphonate treatment is substituted with denosumab therapy. The primary aim of this study is to investigate the 2-year changes in renal function based on serum creatinine or cystatin C levels in women with osteoporosis who initiated denosumab therapy, including those who were switched from long-term bisphosphonate treatment to denosumab therapy. The secondary aim of this study is to investigate whether BMD was increased in patients who were switched from bisphosphonate treatment to denosumab therapy. Our hypothesis was that renal function could be preserved when it was evaluated using serum cystatin C levels instead of serum creatinine levels in both groups and that the BMD may increase when switching from bisphosphonate treatment to denosumab therapy.

2. Methods

2.1. Patients

This retrospective observational study was conducted at Enshu Hospital (Hamamatsu, Shizuoka, Japan). Women with osteoporosis aged > 50 years who had BMD and laboratory data, who initiated denosumab therapy between March 2016 and June 2019, and who completed a 2-year denosumab therapy were included. All patients were divided into 2 groups: the new group, which included patients who had never been treated with any drug for osteoporosis, and the switch group, which included patients who were switched from bisphosphonate treatment to denosumab therapy. Osteoporosis was diagnosed based on the Japanese Society for Bone and Mineral Research criteria [15]. Switching from bisphosphonate treatment to denosumab therapy was dependent on the patient's preference in cases of decreased BMD or new osteoporotic fracture during long-term bisphosphonate treatment. Among patients who chose to switch from bisphosphonate treatment to denosumab therapy, no wash-out period was conducted between treatments. The exclusion criteria were as follows: patients treated with osteoporosis drugs other than bisphosphonates, such as selective estrogen receptor modulators, teriparatide, romosozumab, and estrogen, before initiating denosumab therapy; patients with endocrine disorders including primary hyperparathyroidism; patients with possible causes of secondary osteoporosis, such as poorly controlled diabetes mellitus, alcoholism, and post-gastrectomy; patients with metastatic bone cancer; and patients whose eGFRcr was < 30 mL/min, or those undergoing hemodialysis. Since osteoporosis drug therapy should be initiated as early as possible after fracture, patients with recent fractures were also included. Sixty milligrams of denosumab (Daiichi Sankyo Company, Tokyo, Japan) was administered subcutaneously once every 6 months. Two daily tablets of vitamin D and calcium supplementation (762.5 mg of calcium carbonate, 200 IU of cholecalciferol, and 59.2 mg of magnesium carbonate) were prescribed to avoid hypocalcemia. This study was conducted in accordance with the ethical standards of the Declaration of Helsinki and was approved by the Institutional Review Board of Enshu Hospital, number 2021-08-02. The information presented in this study, including the study aim, is available to the public on the homepage of the Enshu Hospital website, http://k-enshu.ja-shizuoka.or.jp. Each patient had the right to opt out of the study. The requirement for informed consent was waived due to the retrospective nature of the study.

2.2. Data collection

Data on patients' age, height, weight, and past medical history including history of fractures, period since the last fracture, and mobility at initiation of denosumab administration and after 24 months, were obtained from medical records. If denosumab therapy was initiated within 3 months of the most recent fracture (early initiation group), ambulatory ability prior to the fracture was assessed.

BMD was measured at the initiation of denosumab therapy and at 6, 12, 18, and 24 months thereafter. Blood and spot urine samples were obtained simultaneously between 9:00 AM and 11:00 AM without overnight fasting at the time of BMD measurement and immediately before the next denosumab injection. Serum calcium and albumin levels were measured 2–4 weeks after the first injection of denosumab to measure serum-corrected calcium levels. The BMD (g/cm^2) of the L2–4 lumbar spine, femoral neck, and total hip on the left side was measured by dual-energy X-ray absorptiometry (DXA) with a QDR Discovery scanner (Hologic, Inc. Madison, Bedford, MA, USA). If a metal implant was inserted on the left side, the BMD of the right hip was measured. The coefficient of variation (CV) for lumbar, femoral neck, and total hip BMD were described in our previous paper [16]. Serum type 1 procollagen N-terminal propeptide (S–P1NP), urinary N-terminal type I collagen telopeptide (U-NTX), serum total homocysteine (S-Hcy), and cystatin C levels were measured along with routine laboratory examinations. Both S–P1NP and U-NTX levels were measured by enzyme-linked immunosorbent assay (ELISA) (Orion Diagnostica, Espoo, Finland for S–P1NP and Osteomark, Osteox International, Seattle, WA, USA, for U-NTX). The U-NTX level was normalized to urinary creatinine level. The S-Hcy level was measured by high-performance liquid chromatography, with an intra-assay CV of 3% and inter-assay CV of 2% [17]. Serum cystatin C levels were measured using sol particle colloidal immunoassay, in which the within and between-day CV varied from 1.1 to 1.6 and 0.4 to 1.0, respectively [18]. Ccr was estimated using the Cockcroft and Gault methods [19]. eGFRcr and eGFRcys were calculated using the following equations for women recommended by the Japanese Society of Nephrology [20,21]:

$$\text{eGFRcr (mL/min/1.73 m}^2) = 194 \times \text{serum creatinine}^{-1.094} \times \text{age}^{-0.287} \times 0.739$$

$$\text{eGFRcys (mL/min/1.73 m}^2) = [104 \times \text{serum cystatin C}^{-1.019} \times 0.996^{\text{age}} \times 0.929] - 8$$

All immunoassays were performed by SRL Inc. (Tokyo, Japan).

Fragility fractures defined in this study included the hip, forearm, proximal humerus, pelvis, distal tibia, and symptomatic and non-symptomatic vertebral fractures. Mobility before denosumab initiation and 24 months after treatment was classified based on the ambulatory ability criteria proposed by Yonezawa et al [22]. The Charlson comorbidity index (CCI) was calculated to evaluate the degree of comorbidity before injury or denosumab initiation [23].

2.3. Statistical analysis

Data were analyzed using StatView 5.0 (SAS Institute, Cary, NC, USA). Analysis of variance (ANOVA), followed by post-hoc Scheffe's F-test, was used to compare numerical data, and the chi-square test was used to compare categorical variables between groups. Comparisons of related values within and between groups were performed using ANOVA, followed by post-hoc Scheffe's F-test. Multiple linear regression analysis was performed to investigate the factors that affected the changes in eGFRcr and Ccr after the 2-year denosumab therapy. Values are expressed as mean ± standard

deviation (SD) or mean ± standard error (SE). P-values < 0.05 were defined as statistically significant. The a priori required sample size was calculated using the G*Power 3.1.9.3 statistical power analysis software program by comparing our previous data on changes in eGFRcr in women with osteoporosis during 27-month minodronate therapy [16] with annual health check-up data of healthy individuals attending our hospital. The mean changes in eGFRcr during minodronate therapy in 99 postmenopausal women aged 43–93 years (average, 74.5 years) for 27 months was −8.5% with a standard deviation of 11.0%. In contrast, the mean changes in eGFRcr in 268 healthy women aged 71–86 years attending to our hospital for annual health check-ups (average, 74.3 years) for 24 months were −2.8% with a standard deviation of 9.2% (unpublished data). Based on a statistical power (1 - beta error probability) of 80%, a two-tailed alpha error probability of 5%, an effect size *d* of 0.563, and 1:1 group allocation ratio, 51 patients were required for each group.

3. Results

3.1. Background data

In total, 117 women with osteoporosis met the inclusion criteria. The new and switch groups included 53 and 64 patients, respectively. The prior treatment with bisphosphonate in the switch group included oral alendronate, risedronate, and minodronate, with an average duration of 54.7 months (Table 1). More than 90% of patients in the switch group were switched from minodronate or risedronate to denosumab. There were no significant differences in age, body mass index, CCI, mobility status, and laboratory data, except for S–P1NP and U-NTX levels, between the groups. The S–P1NP and U-NTX levels in the switch group were significantly lower than those in the new group due to prior treatment with bisphosphonates. The lumbar BMD was significantly higher in the switch group than in the new group; however, there were no significant differences in femoral neck or total hip BMD between the groups. The prevalence of a history of fragility fracture and recent fracture within 3 months prior to denosumab initiation was significantly higher in the new group than in the switch group. Regarding the history of fragility fracture, a history of hip and vertebral fractures were more common in the new group than in the switch group.

3.2. Bone mineral density and bone metabolic markers

Lumbar BMD increased significantly by 10.8% and 4.3% compared to that at baseline in the new and switch groups, respectively, over 2-year denosumab therapy (Fig. 1). Change of lumbar BMD in the new group was significantly larger than that in the switch group (P < 0.0001 for group comparison). Femoral neck and total hip BMD increased in both groups after 2-year denosumab therapy. There was no significant difference for change of femoral neck or total hip BMD between groups. S–P1NP and U-NTX levels significantly decreased compared to those at baseline in the new group; however, U-NTX levels gradually increased from 18 months in the switch group up to those in the new group (Fig. 2).

3.3. Renal function

eGFRcr decreased by 11.5% compared to that at baseline in the new group (Fig. 3-a). Ccr also decreased by 13.3% and 7.0% compared to that at baseline in the new and switch groups during the 2-year denosumab therapy (Fig. 3-b). There were significant differences for changes of eGFRcr and Ccr between groups (P < 0.001 for group comparison in both eGFRcr and Ccr). However, eGFRcys and S-Hcy levels did not change significantly in either group during the study period (Fig. 3-c and d). To examine the factors associated with changes in eGFRcr and Ccr, multiple linear regression analysis was performed for 2-year percent changes in eGFRcr or Ccr among all 117 subjects adjusted for age, body mass index, eGFRcr and Ccr values at the time of initiation of denosumab therapy, prior bisphosphate treatment, and whether denosumab

Table 1
Baseline characteristics of the new and switch groups.

Variable	New group	Switch group	P-value
No.	53	64	
Age, yr	76.3 (9.4)	76.2 (7.9)	ns
BMI, kg/m^2	20.5 (2.9)	20.7 (3.3)	ns
CCI	0.64 (1.08)	0.41 (0.79)	ns
Lumbar BMD T-score, SD	−2.9 (1.1)	−2.1 (1.8)	0.003
Femoral neck BMD T-score, SD	−2.8 (0.8)	−3.0 (0.6)	ns
Total hip BMD T-score (SD)	−2.4 (0.8)	−2.5 (0.7)	ns
S–P1NP,μg/L	83.8 (48.1)	21.8 (9.9)	< 0.0001
U-NTX, nmol BCE/mmol Cr	75.5 (47.0)	25.4 (15.4)	< 0.0001
S-Hcy, μmol/L	12.4 (5.7)	11.2 (3.8)	ns
S-cystatin C, mg/L	1.00 (0.32)	0.92 (0.20)	ns
eGFRcr, mL/min/1.73 m^2	69.1 (17.9)	66.7 (15.5)	ns
eGFRcys, mL/min/1.73 m^2	70.0 (22.8)	73.4 (17.0)	ns
Ccr, mL/min/1.73 m^2	55.7 (19.3)	53.4 (16.5)	ns
Number of prior fragility fractures	44 (83.0%)	39 (60.9%)	0.008
Vertebral fracture	16 (30.2%)	9 (14.1%)	
Hip fracture	23 (43.4%)	17 (26.6%)	
Other fractures	5 (9.4%)	12 (18.8%)	
Initiation within 3 months after fracture	37 (69.8%)	5 (7.8%)	<0.0001
Mobility Independent/Dependent (% independent)	45/8 (84.9%)	68/6 (90.6%)	ns
Number of prior BP therapy	None	ALN 5 (7.8%)	
		RIS 13 (20.3%)	
		MIN 46 (71.9%)	
Duration of prior BP therapy (months)		54.7 (20.5)	

The numbers without percentages in parenthesis indicate standard deviation.
BMI, body mass index; CCI, Charlson comorbidity index; BMD, bone mineral density; S–P1NP, serum type-I procollagen N-terminal propeptide; U-NTX, urinary N-terminal telopeptide of type I collagen; BCE, bone collagen equivalent; S-Hcy, serum homocysteine; eGFRcr, estimated glomerular filtration rate based on creatinine; eGFRcys, estimated glomerular filtration rate based on cystatin C; Ccr, creatinine clearance; BP, bisphosphonate; ALN, alendronate; RIS, risedronate; MIN, minodronate; ns, not significant.

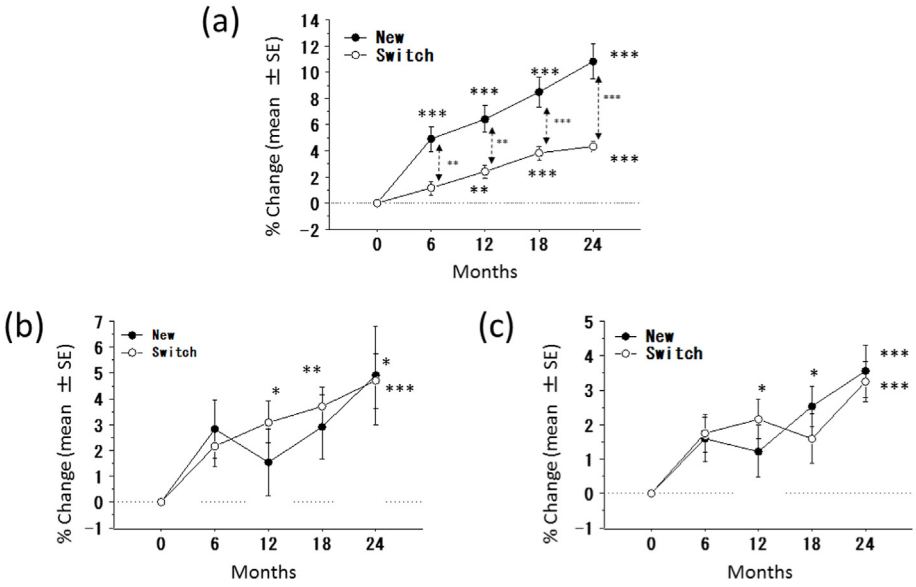

Fig. 1. Percentage changes (mean ± SE) in the lumbar (a), femoral neck (b), and total hip (c) bone mineral density (BMD) during 24 months in the new and switch groups
P <0.05*, P < 0.001**, and P < 0.0001*** compared with baseline values. Bidirectional dotted lines indicate comparison between groups.

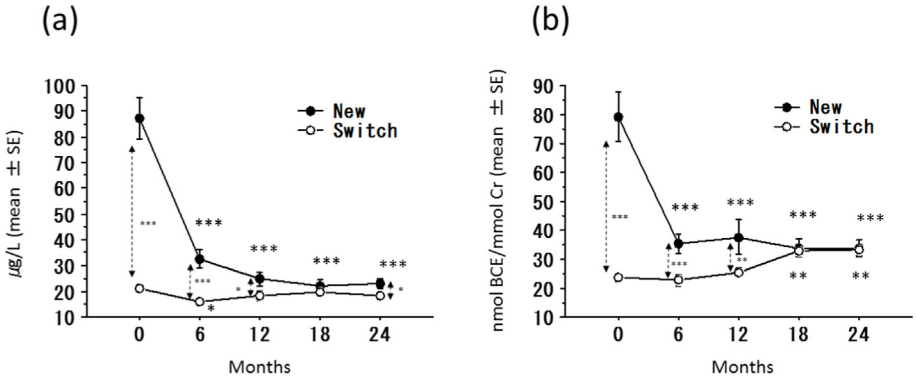

Fig. 2. Changes (mean ± SE) in serum intact procollagen type-I N-terminal propeptide (S–P1NP) (a) and urinary type-I collagen cross-linked N-telopeptide (U-NTX) (b) levels during 24 months in the new and switch groups
P <0.05*, P < 0.001**, and P < 0.0001*** compared with baseline values. Bidirectional dotted lines indicate comparison between groups.

initiation was within 3 months after the fracture (early initiation) as the covariates. Multiple regression analysis revealed that baseline eGFRcr or Ccr and early initiation of denosumab therapy were significant determinant factors for 2-year changes in eGFRcr and Ccr (Table 2). In total, there were 34 patients in the early initiation group, and the remaining 83 patients, in which denosumab was initiated > 3 months after the last fracture or a history of fractures was absent (late initiation or no fracture group). eGFRcr and Ccr decreased more in the early initiation group than in the late initiation or no fracture group (P < 0.001 for group comparison in both eGFRcr and Ccr) (Fig. 4). A greater proportion of the patients were independent in daily living activities in the late initiation or no fracture group than in the early initiation group, both before and at the end of the study (Table 3). Although all subjects had recent fragility fracture in the early initiation group, 88.5% (23 of 26 patients) of patients who were independent before fracture recovered their mobility to the pre-fracture level during the 2-year denosumab therapy.

4. Discussion

The present study demonstrated that the renal function based on cystatin C was not affected by 2-year denosumab therapy, even in patients with prior long-term bisphosphonate use. However, changes in renal function based on serum creatinine levels, such as those in eGFRcr and Ccr, were affected by recent fractures within 3 months prior to denosumab initiation. BMD increased significantly in patients who were switched from long-term bisphosphonate treatment to denosumab therapy.

It is surprising to note that there was no significant difference at baseline in renal function, including eGFRcr, Ccr, eGFRcys, and S-Hcy levels, between the new and switch groups despite an average 54.7-month oral bisphosphonate treatment in the switch group. According to a review paper by Miller et al [24], oral bisphosphonate therapy for up to 2 years, including alendronate, risedronate, and ibandronate, did not result in degradation of renal function. Our data indicated that > 3-year oral bisphosphonate treatment

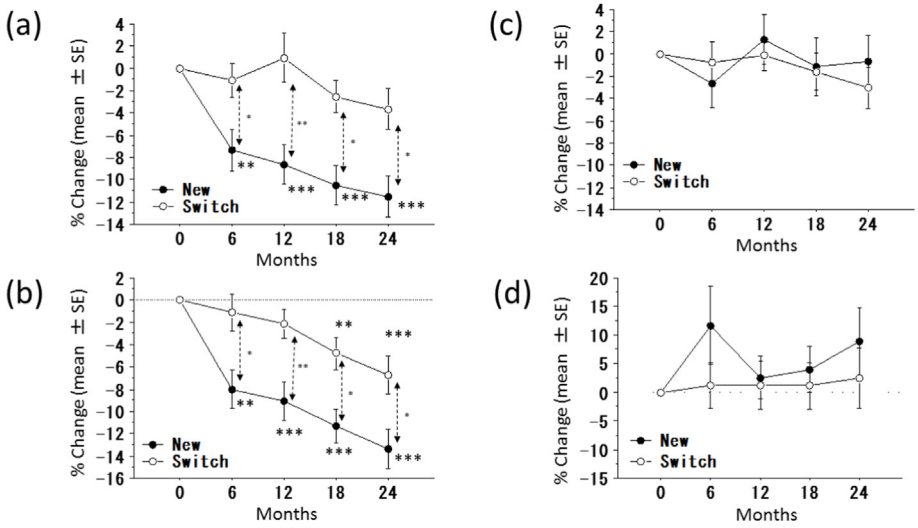

Fig. 3. Percentage changes (mean ± SE) in eGFRcr (a), Ccr (b), eGFRcys (c), and S-Hcy level (d) during 24 months in the new and switch groups
P <0.05*, P < 0.001**, and P < 0.0001*** compared with baseline values. Bidirectional dotted lines indicate comparison between groups.

Table 2
Multiple linear regression of percent changes in eGFRcr and Ccr over 2 years with variables.

	24-month eGFRcr change			24-month Ccr change		
Variable	β	P-value	95% CI	β	P-value	95% CI
Age, yr	−0.111	0.268	−0.481 to 0.137	−0.338	0.017	−0.912 to −0.090
BMI, kg/m^2	−0.051	0.531	−0.927 to 0.481	0.147	0.141	−0.200 to 1.396
Initial eGFRcr or Ccr, mL/min/1.73 m^2	−4.456	<0.0001	−0.495 to −0.190	−0.534	<0.001	−0.582 to −0.178
Prior BP therapy (yes/no)	0.861	0.392	−3.209 to 8.139	0.484	0.629	−4.087 to 6.729
Initiation within 3 months after fracture (yes/no)	−2.483	0.015	−14.954 to −1.680	−0.309	0.008	−14.920 to −2.290

eGFRcr, estimated glomerular filtration rate based on creatinine; Ccr, creatinine clearance; BMI, body mass index; BP, bisphosphonate.

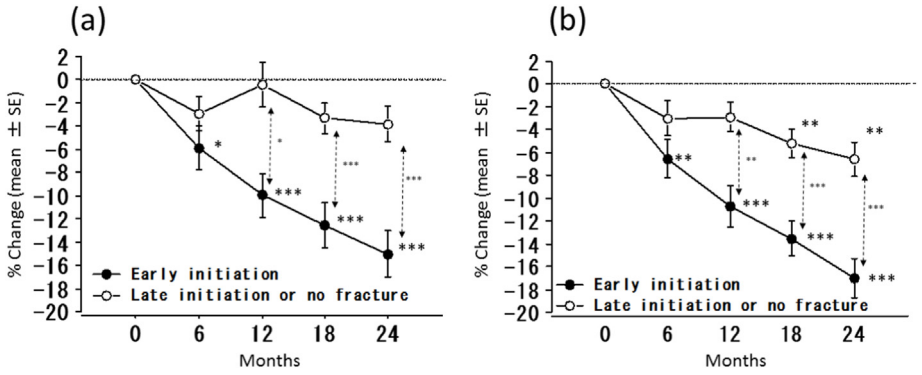

Fig. 4. Percentage changes (mean ± SE) in eGFRcr (a) and Ccr (b) during 24 months in patients who initiated treatment within 3 months after fracture (early initiation) and those who initiated treatment ≥3 months after fracture or those who did not have a fracture (late initiation or no fracture).
P < 0.05*, P < 0.001**, and P < 0.0001*** compared with baseline values. Bidirectional dotted lines indicate comparison between groups.

Table 3
Mobility before and 2 years after denosumab therapy.

	Early initiation (N = 34)	Late initiation or no fracture (N = 83)	P-value
Number of patients with prior fragility fracture	34 (100%)	49 (59.0%)	<0.0001
Before treatment			
Independent/Dependent	26/8	77/6	0.019
(% independent)	(76.5%)	(92.8%)	
After treatment			
Independent/Dependent	23/11	76/7	0.002
(% independent)	(67.6%)	(91.6%)	

Early initiation, initiation within 3 months after fracture.
Late initiation or no fracture, initiation 3 months or more after fracture or without fracture.

may not have an impact on renal function either.

In several studies, BMD increased when switching from oral bisphosphonate to denosumab in clinical practice; however, most studies only investigated BMD in the short term or for approximately 1 year [25–28]. Indeed, studies conducted over a longer period (2 years or more) are scarce [29,30]. The increase in BMD after switching from bisphosphonate treatment to denosumab may account for the different mechanisms of action of the 2 drugs. Bisphosphonates are incorporated into the bone and are absorbed by osteoclasts, leading them to death. On the other hand, denosumab disturbs maturation of preosteoclasts and promotes malfunctioning of mature osteoclasts [31]. Denosumab therapy has a stronger anti-resorption efficacy than oral bisphosphonate as it exerts osteoclasts in a wider maturation stage than bisphosphonates. In our study, U-NTX levels in the switch group increased to the same levels as those in the new group at the end of the study. A recent experimental animal study demonstrated that minodronate had approximately the same degree of affinity to hydroxyapatite as that of risedronate, which is considered to have a lower affinity to the bone than alendronate and zoledronate [32]. In our study, > 90% patients in the switch group had been receiving minodronate or risedronate. Bisphosphonates such as minodronate and risedronate have lower affinity to hydroxyapatite and appear to be retained in the bone for a shorter period compared to alendronate and zoledronate. The exact retention period of minodronate and risedronate in the human bone is unclear; however, the effects of bisphosphonate treatment may start to subside at 18 months after therapy cessation.

Serum cystatin C is superior to creatinine in detecting subtle changes in renal function in older adults [33]. Miyaoka et al [14] reported that renal function was improved by 2-year denosumab therapy, evaluated using eGFRcys in 73 men and women with osteoporosis, possibly due to the decrease in serum phosphate levels by denosumab. In our study, we did not observe improvement in renal function using the same marker for kidney function as that used in the previous study; however, our results showed that renal function was not affected by 2-year denosumab therapy, even in patients who received long-term treatment with bisphosphonate. Steady S-Hcy levels in both groups during the 2-year period also supported this conclusion. Homocysteine is an indicator of renal function and atherosclerosis. S-Hcy levels are significantly correlated with renal function and are increased in the early phase of renal failure [34,35].

Post-hoc analysis of a controlled randomized trial on denosumab (FREEDOM study) revealed that renal function, assessed using eGFRcr, was preserved during > 10-year denosumab therapy. However, 20% of patients with CKD stage 2 at baseline were re-categorized to stage 3 after 10 years and 20% patients were re-categorized from stage 3a to stages 3b and 4 [13]. This could be partly attributed to the decline in kidney function due to normal aging. However, in daily clinical practice, a decrease in eGFRcr is occasionally encountered during denosumab therapy among patients with various backgrounds, including those with recent fractures, those with older age, or those with poor renal function. In our study, the decline in eGFRcr and Ccr in the switch group was 3.7% and 7.0% (Fig. 3-a, -b), respectively, compared to those at baseline. For patients in the late initiation and no fracture group, the decline in eGFRcr and Ccr was 3.8% and 6.6%, respectively (Fig. 4). Based on post-hoc analyses of the Health Outcomes and Reduced Incidence with Zoledronic Acid Once Yearly Pivotal Fracture Trial, which was a 3-year randomized controlled trial on treatment with 5 mg zoledronic acid once a year for postmenopausal women with osteoporosis, time-dependent changes in Ccr calculated using the Cockcroft–Gault equation in 2514 patients in the placebo group over 3 years was shown. The decline in Ccr after 2 years was approximately 6% compared to that at baseline [36]. There was an identical change in Ccr in the switch group and the late initiation or no fracture group in our study, indicating age-related deterioration of renal function.

eGFRcr and Ccr decreased significantly in the new group during the study period. Multiple linear regression analysis suggested that recent fracture within 3 months prior to denosumab initiation was an independent factor for the 2-year decline in eGFRcr and Ccr. The decline in eGFRcr and Ccr in the new group could be affected by the background characteristics related to the history of recent fractures. Approximately 70% of patients in the new group experienced a recent fracture within 3 months prior to denosumab initiation, in contrast to 7.8% in the switch group. The decrease in eGFRcr and Ccr in the early initiation group may have been due to the increase in muscle mass resulting from improved mobility during the recovery process after a recent fracture; > 70% recent fractures were hip or vertebral fractures. In a study by Fischer et al [37], muscle strength of the lower extremities increased in the first 6 months, and subjective physical functioning continued to increase up to 9 months after hip fracture in older adults. In a systematic review article, approximately 61% of patients with hip fracture achieved pre-hip fracture levels of activity or health outcomes during the 2-year recovery period after fracture [38]. In our study, 88.5% (23/26) of patients who initiated denosumab treatment within 3 months after fracture recovered to the pre-fracture level within 2 years (Table 3). Ongoing muscle recovery accompanied by expansion of daily activities after fracture during the 2-year denosumab therapy may have resulted in a decrease in eGFRcr or Ccr due to increased serum creatinine levels (data not shown). There is an inverse relationship between serum creatinine levels and eGFRcr or Ccr levels. Based on our results, the values of eGFRcr or Ccr during denosumab therapy should be carefully interpreted in patients with recent fractures to avoid underestimation of renal function. More studies are needed to elucidate the relationship between the decrease in eGFRcr and muscle mass, such as measuring lean body mass or estimated muscle volume by image inspection immediately after a fracture.

Our study has several limitations. First, this was a retrospective, observational study and not a prospective study. The background data in both groups were not identical regarding the prevalence of previous fractures and the timing of denosumab initiation after fracture. In the present study, most of the subjects in the new group were diagnosed with osteoporosis only when fragility fracture was confirmed at our hospital and denosumab was started as early as possible. Thus, more subjects in the new group had prior fragility fracture compared to those in the switch group. Moreover, long-term bisphosphonate therapy may contribute to lower prevalence of prior fracture in the switch group. Furthermore, mobility status before denosumab treatment was different between the early initiation group and the late initiation or no fracture group. According to Pluijm et al [39], mobility impairment was one of the risk factors for occurrence of new fragility fracture by prospective cohort study. It is suggested that patients in the early initiation group which included all the subjects with recent fragility fracture had been susceptible to fracture by nature due to mobility impairment (Table 3). Secondly, the number of patients was relatively small; however, the a priori calculated sample size was sufficient to verify the statistics. Finally, actual muscle volume was not measured to assess the relationship between eGFRcr or Ccr and muscle mass.

5. Conclusions

Denosumab did not have an adverse effect on renal function during the 2-year treatment period in women with osteoporosis, even in those with a history of long-term bisphosphonate

treatment. Decreased eGFRcr and Ccr may be reflected by increased muscle volume during the recovery phase after a recent fracture. More BMD gain can be expected with denosumab therapy in women with osteoporosis who switched from long-term bisphosphonate treatment to denosumab therapy.

CRediT author statement

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Conflicts of interest

The authors declare no competing interests.

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