J Orthop Sci (2011) 16:418–423
DOI 10.1007/s00776-011-0089-4

ORIGINAL ARTICLE

Serum 25-hydroxyvitamin D status in hip and spine-fracture patients in Japan

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Received: 29 September 2010 / Accepted: 12 April 2011 / Published online: 19 May 2011

Abstract
Background Serum 25-hydroxyvitamin D (25(OH)D) is used as an index that reflects the level of vitamin D. We have previously reported, on the basis of a study in Sado in Niigata, that patients with hip fracture have lower serum 25(OH)D levels than non-hip-fracture cases. In this study, the serum 25(OH)D status in hip-fracture cases was examined in four regions in Japan. Although most hip-fracture patients have experienced past spine-compression fractures, the relationship of these fractures and 25(OH)D is unknown. Therefore, we also examined the 25(OH)D level in spine-compression fracture patients in the same locations and time periods.
Methods The levels of 25(OH)D, intact parathyroid hormone (intact PTH), undercarboxylated osteocalcin (ucOC), urine *N*-terminal crosslinking telopeptide of type I collagen (NTX), and bone mineral density were examined in patients with hip and spine fracture due to osteoporosis in several regions in Japan.
Results There were no significant differences in age, BMI, serum 25(OH)D, serum intact PTH, and serum ucOC among the regions. Levels of serum 25(OH)D were low in patients with hip fracture and spine fracture. The average serum 25(OH)D level was significantly lower in hip-fracture patients than in spine-fracture patients (16.3 vs. 18.1 ng/mL, $P < 0.05$). High serum ucOC was found in 37% of hip-fracture patients and 44% of spine-fracture patients.
Conclusions Both hip and spine-fracture patients have vitamin D insufficiency, with similar results found in elderly patients in four areas of Japan. The severity of this condition tends to be more serious in hip-fracture patients than in spine-fracture patients.

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Introduction

Osteoporosis causes fractures, serious physical and mental damage, and decreased activities of daily living (ADL) and quality of life (QOL). Hip fractures and vertebral compression fractures are especially common in elderly people [1, 2], and the negative effects of these fractures on ADL and QOL emphasize the need to determine the associated

Table 1 Number of patients in the study

	Hip fracture					Spine fracture				
	Sado	Aichi	Tottori	Kumamoto	Total	Sado	Aichi	Tottori	Kumamoto	Total
Male	14	11	12	15	52	4	6	2	0	12
Female	52	37	26	58	173	27	14	11	0	52
Total	66	48	38	73	225	31	20	13	0	64

risks and causes and to establish preventive measures. A relationship between serum vitamin D (25(OH)D; 25-hydroxyvitamin D) level and hip fracture has been established. Overseas [3, 4] and domestic reports, including an epidemiologic survey in Sado City in Niigata Prefecture in 2004 [5], have shown that 25(OH)D is significantly lower in hip-fracture patients than in controls. Furthermore, half of Japanese women aged >65 years old also have insufficient levels of serum 25(OH)D [6, 7], and this may be a major risk factor for hip fracture.

Epidemiologic surveys suggest that the incidence of hip fracture is lower in Japan than in Europe and the United States [8–10], and there are also regional differences in Japan. Furthermore, because most hip-fracture patients (81.8%) have past vertebral compression fracture on X-ray [5], the relationship of compression fracture and serum 25(OH)D is of concern. In this study, the relationship between serum 25(OH)D and hip fracture was examined from a perspective of regional differences in Japan. We also aimed to clarify the relationship between spine fracture and serum vitamin D level, and to examine the vitamin K status of patients with hip fracture or spine fracture.

Patients and methods

Study site

A survey of patients treated for hip fracture and spine-compression fracture was performed in one or two hospitals in several areas of Japan: Niigata (Sado), Aichi, Tottori, and Kumamoto prefectures.

Subjects

The subjects were inpatients and outpatients aged ≥65 years old with fresh hip and spine-compression fracture treated from April 1, 2007 to March 31, 2008. All patients gave consent to the study. For compression fracture, it was not always easy to identify a new fracture. However, patients who visited the hospital for symptoms such as back pain and were judged, on the basis of X-ray and physical examination by an orthopedic doctor, to have a fresh vertebral fracture were considered as a case of new fracture (an incident of fracture: clinical fracture).

There were 102, 81, 57, and 90 subjects from Sado, Aichi, Tottori, and Kumamoto, respectively. Of these 330 patients, 16 with a tumor, osteomalacia, bone fracture due to systemic diseases, hyperthyroidism, hyperparathyroidism, renal failure, or dialysis were excluded. This left 314 patients (66 males, 247 females, 1 unknown) for whom data were collected. Of these patients, data were analyzed for 289 (225 cases of hip fracture and 64 of spine fracture; Table 1) after exclusion of patients who had taken drugs such as active vitamin D, vitamin K, and bisphosphonate, and one patient of unknown sex.

There were more patients with hip fracture than with spine-compression fracture in this study. Epidemiologically, there were more patients with spine fracture than hip fracture, but those with spine fracture were mainly outpatients. This reduced the number of cases of spine fracture in the analysis, and there was no selective exclusion of spine-fracture patients in the study.

Measurements

Data were collected for body height and weight (body mass index, BMI), serum 25(OH)D, serum intact PTH (intact parathyroid hormone), urine NTX (*N*-terminal crosslinking telopeptide of type I collagen), serum undercarboxylated osteocalcin (ucOC), bone mineral density (BMD) in the hip, and history of fractures of other bones, including the spine, hip, distal radius, and proximal humerus. Blood samples for biochemical assays were collected within 1 week after fracture. The exact date of spine fracture was often uncertain, but most data were collected within 1 week after the first medical examination.

The serum 25(OH)D level was measured by enzyme-linked immunosorbent assay (ELISA) assay using a kit supplied by DiaSorin (Stillwater, MN, USA). A serum 25(OH)D level of at least 15–20 ng/mL is needed to optimize PTH levels, on the basis of several reports. Hollis et al. [11] found that the normal range of 25(OH) D was 32–100 ng/mL and that a concentration of <10 ng/mL indicated a vitamin D-deficient state. Other studies performed in the USA and Australia [12, 13] show that a serum 25(OH) D level of at least 15–20 ng/mL is needed to

achieve an optimum PTH level, and therefore we defined a 25(OH) D level of <20 ng/mL as vitamin D insufficiency.

Serum-intact PTH was measured by means of an electrochemiluminescence immunoassay (ECLIA) (Roche Diagnostics, Basel, Switzerland), in which intact PTH molecules are detected; the normal range is 10–65 pg/mL [14, 15]. We note that Segersten et al. [16] have suggested that the upper limit of the normal range for PTH may be too high; however, LeBoff et al. [4] used a value of 65 pg/mL, and we also chose 65 pg/mL as the upper limit of the normal range for intact PTH.

The urine NTX assay was performed using an Osteomark NTX ELISA kit (Inverness Medical Professional Diagnostics, Princeton, NJ, USA). Serum ucOC was measured by ECLIA (Sanko Junyaku, Tokyo, Japan). A high level of serum ucOC is a reported risk factor for hip fracture [17, 18]. In patients with vitamin K insufficiency, osteocalcin (OC) (a basic bone protein produced by osteoblasts) is released into blood as ucOC, which has a glutamic acid (Glu) residue that is not converted to a γ-carboxyl glutamate. This reduces OC incorporation into bone. The cutoff value for serum ucOC is 4.5 ng/mL [19, 20].

BMD of the hip was measured by dual-energy X-ray absorptiometry (DXA) (in Sado: Hologic 4500A, Bedford, MA, USA; in Aichi: DPX-NT; GE Medical Systems Lunar, Madison, WI, USA; in Kumamoto: Hologic Delphi, Bedford, MA, USA). In hip-fracture cases, BMD was measured in the hip on the opposite side to the fractured hip. Data for past fractures of the hip, spine, distal radius, and proximal humerus were determined by interview or X-ray.

Statistical analysis

Comparison between two groups was performed using a non-paired t test for parametric variables and a Mann–Whitney U test for non-parametric variables. Comparison among multiple groups was performed using ANOVA, followed by a Tukey test for parametric variables and a Kruskal–Wallis test for non-parametric variables. Analysis was performed using Microsoft Excel 2007 and Ekuseru Toukei 2008 for Windows.

Ethical considerations

The study plan was approved by the Japanese Orthopedics Association Ethical Review Board. The study was explained in writing to the patients and informed consent was obtained.

Results

Data were collected for 66 cases of hip fracture (52 females and 14 males) and 31 of spine-compression fracture (27 females and 4 males) in Sado City, Niigata (an island city) (Table 1); for 48 cases of hip fracture (37 females, 11 males) and 20 of spine fracture (14 females and 6 males) in Aichi Prefecture (National Center for Geriatrics and Gerontology); for 38 cases of hip fracture (26 females and 12 males) and 13 of spine fracture (11 females and 2 males) in Tottori Prefecture (including patients in three hospitals); and for 73 cases of hip fracture (58 females and 15 males) and 0 of spine fracture in Kumamoto Prefecture (Tamana Central Hospital).

Data in the four regions

The average values of variables in each region are shown in Table 2. The average age at the time of injury ranged from 82 to 84 years old for hip-fracture cases, with no significant differences among the regions. For BMI in hip-fracture patients also there were no significant differences among regions. The order of BMI in spine fracture was Tottori > Sado > Aichi, with no significant differences among regions. The average levels of serum 25(OH)D (<17 ng/mL) in hip-fracture patients were low in all four areas. These values were especially low in Sado and Aichi, but there were no significant differences among the regions. The mean serum 25(OH)D level was 17–19 ng/mL in spine-fracture cases, and was lowest in Sado, but again with no significant regional differences.

The average serum intact PTH level (>45 pg/mL) in hip-fracture patients was comparatively high in all four areas, with no significant regional differences. This level ranged from 40 to 47 pg/mL in spine-fracture patients, and there were also no significant differences among the areas.

In patients with hip fracture, urine NTX was significantly higher in Aichi and Sado than in Kumamoto ($P < 0.01$ and $P < 0.05$, respectively). There were no significant differences in urine NTX in spine-fracture patients among the regions.

Data for serum ucOC were collected from Sado, Tottori, and Kumamoto, and showed no significant differences among these areas. BMD analysis was performed in Sado, Aichi, and Kumamoto. Because radial and spine BMD were measured in Tottori, we excluded these data from the analysis. BMD in hip-fracture patients in Sado was significantly lower than that in Aichi ($P < 0.001$). There were no significant differences in BMD in spine-fracture patients among the regions.

Comparison of hip and spine fracture

A comparison of variables in hip and spine-fracture cases is shown in Table 3. The average age over all regions was significantly higher for hip fracture than for spine fracture (83.0 vs. 80.1 years old, $P < 0.01$). BMI showed no

Table 2 Average values of variables for cases of hip and spine fracture in each region (mean ± SD)

	Variables/ Regions	Age (years old)	BMI (kg/m²)	Serum 25(OH)D (ng/mL)	Serum intact PTH (pg/mL)	Urine NTX-cre (nmol BCE/nmol Cr)	Serum ucOC (ng/mL)	BMD (g/cm2)
	Sado	84.3±7.83	20.1±3.34	16.0±5.61	55.0±53.6	98.6±52.5	4.48±4.49	0.465±0.164
Hip fracture	Aichi	82.2±9.38	19.6±4.01	15.5±4.88	46.8±19.04	107.6±63.8	-	0.585±0.144
(*n*=225)	Tottori	83.2±8.07	21.2±3.64	17.1±5.41	46.3±23.6	84.0±46.2	4.57±3.34	-
	Kumamoto	82.3±11.5	20.2±3.05	16.9±4.48	59.7±31.5	73.7±42.5	4.61±4.50	0.535±0.140
	Sado	79.6±6.67	21.8±5.56	17.5±6.00	47.3±18.1	76.8±46.8	6.61±5.59	0.522±0.164
Spine fracture	Aichi	81.7±5.85	20.3±6.04	19.2±5.05	41.9±22.1	92.8±44.4	-	0.590±0.203
(*n*=64)	Tottori	79.2±5.85	22.4±2.49	17.7±5.5	43.7±17.0	67.7±26.2	5.18±2.83	-
	Kumamoto	-	-	-	-	-	-	-

* $P < 0.05$, ** $P < 0.01$

Table 3 Average values of variables for cases of hip and spine fracture

Variables	Hip fracture	Spine fracture	*P* value
Age (years old)	83.0 ± 9.51	80.1 ± 6.26	$P < 0.01$
BMI (kg/m²)	20.5 ± 3.49	21.4 ± 5.15	n.s.
Serum 25-OHD (ng/mL)	16.3 ± 5.13	18.1 ± 5.59	$P < 0.05$
Serum intact PTH (pg/nL)	53.3 ± 36.8	44.9 ± 19.1	n.s.
Urine NTX (nmol BCE/nmol Cr)	89.9 ± 53.5	80.5 ± 42.9	n.s.
Serum ucOC (ng/mL)	4.55 ± 4.25	6.18 ± 4.95	$P < 0.01$
BMD (g/cm²)	0.521 ± 0.163	0.616 ± 0.136	$P < 0.01$

significant difference between hip and spine cases, but the average BMI in spine-fracture patients tended to be higher than that in hip-fracture patients. The average 25(OH)D level in hip-fracture patients was significantly lower than that in spine-fracture patients (16.3 vs. 18.1 ng/mL, $P < 0.05$). There were no significant differences in intact PTH between hip and spine-fracture cases, but the average intact PTH in hip-fracture patients tended to be higher than that in spine-fracture patients.

Urine NTX was elevated in both fracture types, with no significant difference between the two types. The average serum ucOC level was significantly lower in patients with hip fracture than in those with spine fracture (4.55 vs. 6.18 ng/mL, $P < 0.01$). BMD was low for both types of fracture, and mean BMD for all hip-fracture cases was significantly lower than that for all spine-fracture cases (0.521 vs. 0.616 mg/cm², $P < 0.001$).

The percentages of patients with 25(OH)D <20 ng/mL were 79.0% for hip-fracture cases and 66.7% for spine-fracture cases (Fig. 1). Data for ucOC were available for Sado, Tottori, and Kumamoto. In these regions, the percentages of patients with ucOC ≥ 4.5 ng/mL were 36.2% in hip-fracture cases and 50.0% in spine-fracture cases (Fig. 2).

Past fractures

For evaluation of past fracture, asymptomatic spine-compression fracture was evaluated on the basis of X-ray only in Sado. This analysis showed that 83.3% of hip-fracture patients had past fracture. These data in other areas were obtained by interview, and indicated that 16.7–20.5% of hip-fracture patients had past fractures (Table 4). The percentage of patients with past fracture among spine-fracture patients ranged from 12.9 to 25.0%. Past spine-compression fracture was most common in both hip and spine-fracture patients. Because the data range was wide and there was a large difference between the fractures counted by interview and those assessed by X-ray, including asymptomatic

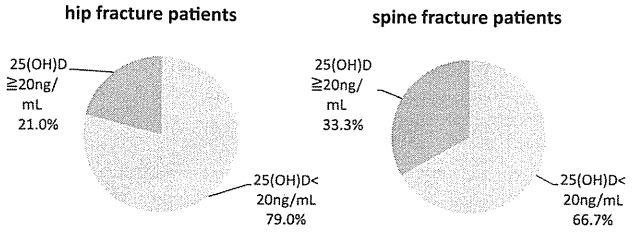

Fig. 1 Percentages of patients with hip or spine fracture with high and low serum 25-OHD levels. The percentages of patients with 25(OH)D < 20 ng/mL were 79.0% in hip-fracture cases and 66.7% in spine-fracture cases

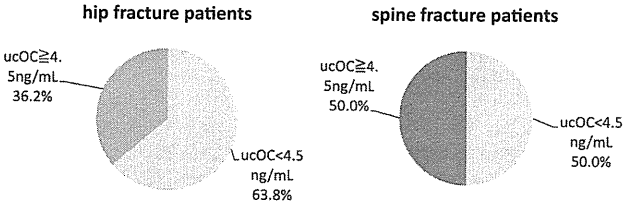

Fig. 2 Percentages of patients with hip or spine fracture with high and low serum ucOC levels. The percentages of patients with ucOC ≥ 4.5 ng/mL were 36.2% in hip-fracture cases and 50.0% in spine-fracture cases

Table 4 Numbers of patients who had past fracture

Area	Patients with hip fracture *N* (%)	Patients with spine fracture *N* (%)
Sado	55[a] (83.3)	4 (12.9)
Aichi	8 (16.7)	5 (25.0)
Tottori	7 (18.4)	3 (21.4)
Kumamoto	15 (20.5)	–

N, number of patients who had past fractures (spine, hip, distal radius, and proximal humerus)

[a] Asymptomatic past spine-compression fracture was assessed by X-ray in Sado. Other data were obtained by interview

compression fracture, we concluded that accurate information on past fractures cannot be obtained by interview.

Discussion

Serum 25 (OH)D and ucOC status

The serum 25(OH)D was low in both hip and spine-fracture patients in all four areas (<20 ng/mL). Intact PTH was slightly elevated in both fracture types and all areas. Low 25(OH)D (vitamin D insufficiency) leads to a high level of intact PTH, indicating slight secondary hyperparathyroidism.

Serum 25(OH)D differences caused by changes in daylight hours at different latitudes are thought to affect the incidence of hip fracture, but this study showed no marked regional differences for either fracture type. However, because data from Northern Japan were not included in this study, it is unclear whether there is any regional difference in an area of higher latitude than Sado.

Fewer fermented soybeans (Natto) are consumed in Western Japan than in the Eastern part of the country [21], and ucOC levels can be viewed in this context. However, there were no significant regional differences in these levels in this study.

Comparison of hip and spine fracture

We also examined differences between hip and spine fractures. The average age at the time of injury was 2.4 years older for hip-fracture cases than for spine-fracture cases ($P < 0.01$). Because approximately 80% of patients with hip fracture also have spine fracture [5], this suggests a chain of events of vitamin D insufficiency → bone absorption acceleration → spine fracture → hip fracture.

The 25(OH)D level was lower ($P < 0.05$) and intact PTH tended to be higher (N.S.) in hip-fracture patients than in spine-fracture patients (Table 3). Low 25(OH)D was more common in hip fracture, and almost two-thirds of spine-fracture patients also had low 25(OH)D (Fig. 1). These results indicate that vitamin D insufficiency and resulting slight hyperparathyroidism were present in patients with both kinds of fracture. These conditions were more severe in hip-fracture patients, which is consistent with the chain of events described above.

The ucOC level was higher in spine fracture than in hip fracture ($P < 0.01$) (Table 3). High ucOC was found in half of the spine-fracture patients, but only one-third of the hip-fracture patients (Fig. 2). That is, vitamin K deficiency was more serious in spine-fracture patients than in hip-fracture patients. However, other factors tended to be more severe in hip-fracture patients. This contradictory result might be because of a change in the serum ucOC level in the period after fracture and before measurement. Blood samples may not always have been collected within 1 week after fracture in spine-fracture cases, because it was not always clear when the fracture had occurred. Therefore, we cannot exclude the possibility of a change in the serum ucOC level in the period after fracture.

Tsugawa et al. [22] reported that the incidence of vertebral fracture in patients with a low plasma phylloquinone (K_1) concentration was significantly higher than that in those with a high K_1 level. However, the ucOC level has not been compared between cases of hip and spine fracture, and clarification of this issue requires further study.

This study was performed in several areas across Japan. The results indicated that differences between hip and

spine fracture were more significant than regional differences. We note that our data do not cover the entire country and further studies of regional differences are required. However, there are few spine-fracture cases in some regions and values for BMD and ucOC are not available in some areas, which may prevent complete analysis. Within this limitation, our results show that both hip and spine-fracture patients have vitamin D insufficiency, which is a risk factor for fracture, based on measurement of serum 25(OH)D and other factors in elderly patients in four areas of Japan. The severity of this condition was more serious in hip-fracture patients.

Acknowledgments We thank the orthopedic departments of the institutions that contributed to this study. We are especially grateful to Drs M Takemura, Y Terabe and T Hida, National Center for Geriatrics and Gerontology, Dr M Shimizu, Shimizu Hospital, Dr M Nakashima, Nojima Hospital, and Drs A Hattori and T Oinuma, Sado General Hospital, for their cooperation. The study could not have been completed without the assistance of all the orthopedic surgeons and numerous other health-care professionals. We express our gratitude to all the individuals who assisted with the study. We also thank Dr Naohito Tanabe, Department of Public Health, Niigata University Graduate School of Medical and Dental Sciences, for advice on statistical analysis. This study was funded by the Committee on Osteoporosis of the Japan Orthopedic Association (we especially thank Drs K Sakamoto, E Ihi, K Aoyagi, K Kita, K Yamazaki, N Yamamoto, T Nakamura, and R Toyoshima) and partially supported by a grant-in-aid from the Ministry of Health, Labor and Welfare of Japan (grant H18-Choujyu Ippann-036).

Conflict of interest The authors did not receive and will not receive any benefits and funding from any commercial party related directly or indirectly to the subject of this article.

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