

Lower osteocalcin and osteopontin contents of the femoral head in hip fracture patients than osteoarthritis patients

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Abstract

Summary In patients with femoral neck fracture, clinical factors, bone metabolism markers (in serum, urine, and bone), bone mineral density, radiographic parameters, and bone histomorphometric parameters were investigated to detect determinants of fragility fracture. The osteocalcin/deoxypyridinoline ratio and osteopontin/calcium ratio of cortical bone were selected as significant predictors.

Introduction Measurement of bone mineral density is widely used to assess bone strength, but this also depends on other bone components and on bone structure. The objective of this study was to investigate risk factors for fracture related to bone quality, the patient's history, and the patient's lifestyle.

Methods Twenty-one patients with femoral neck fracture and 18 patients with osteoarthritis were enrolled. Blood and urine samples were collected on admission to

hospital, and bone samples were obtained from femoral necks resected during surgery. Multivariate logistic regression analysis was performed using osteoarthritis and femoral neck fracture as combined variables to assess the influence of alcohol or coffee intake, eating natto (fermented soybeans), osteocalcin and calcium concentrations, the osteocalcin/deoxypyridinoline ratio and osteopontin/calcium ratios of cortical bone and cancellous bone, various bone histomorphometric parameters, the bone mineral density of the lumbar spine and the intact contralateral femoral neck, and various radiographic parameters of the spine

Results By forward stepwise multivariate analysis, the osteocalcin/deoxypyridinoline and osteopontin/calcium ratios of cortical bone were selected as significant factors for fracture (the odds ratios were 0.493 and <0.001, respectively; both $P < 0.001$).

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Conclusions A decrease of osteopontin and osteocalcin in bone is important for promoting vulnerability to hip fracture.

Keywords Bone mineral density · Fracture risk · Histomorphometry · Multivariate analysis

Introduction

At present, the risk of fracture is evaluated in two different ways. One method involves assessing the strength of the bones from parameters, while the other method is to evaluate the risk of fracture based on the history of each patient [1–3].

The strength of bone is dependent on four factors, which are the bone mineral density, bone structure, bone matrix, and accumulation of microcracks [4–16]. However, there are no methods for direct measurement of the bone matrix or microcracks *in vivo*. Bone structure is not often assessed because of radiation exposure and lack of suitable equipment. Therefore, in clinical practice, bone mineral density is commonly used as the indicator of bone strength. However, research performed to date has shown that it is difficult to completely explain the risk of fracture on the basis of the bone mineral density [17, 18].

The concept of fracture risk has recently been employed in epidemiological surveys [19, 20]. This refers to the risk of fracture occurring in a particular patient, and not only the bone mineral density but also the past history and lifestyle factors are taken into consideration when comparing patients with or without fragility fractures. However, the clinical evaluation of fracture risk is still heavily influenced by bone mineral density, since assessment of bone strength depends on the measurement of bone mineral density and current therapy to reduce fracture risk focuses on increasing bone mineral density. Instead, we considered that evaluation of fracture risk should not be based solely on measurement of bone mineral density and should also include other factors related to bone structure and the bone matrix.

Accordingly, we conducted a survey of patients who had femoral neck fractures and patients who had osteoarthritis with the objective of clarifying the risk of fracture based on the bone mineral density, bone matrix, and bone structure, as well as the patient's past history. Bone metabolism markers obtained from biochemistry tests and urinalysis, degenerative changes on X-ray films of the spine, the bone mineral density of the femoral neck and lumbar spine, histomorphometric parameters of the femoral head, markers of osteoblast growth, and typical items from the past history and lifestyle factors were investigated in order to identify factors that contributed to the risk of fracture.

Materials and methods

Study design

The subjects were postmenopausal women who underwent hemi or total hip replacement because of femoral neck fracture due to osteoporosis or osteoarthritis of the hip at five hospitals during the period from April 2006 to March 2007. The subjects gave written informed consent to participation after receiving an adequate explanation of the study. An interview (past history and lifestyle factors) was performed, along with blood and urine sampling, bone densitometry, and spinal radiography. The femoral heads and necks that were resected during hip surgery were collected and sectioned. Some of the sections were used for analysis of the bone protein content and others were employed for bone histomorphometry. This study was performed with the approval of the Ethics Committee of University of Occupational and Environmental Health (Kitakyushu, Japan).

Subjects

The subjects were all postmenopausal women over 50 years of age who fitted into either of the groups below among those hospitalized for femoral head replacement.

- (1) Patients undergoing femoral neck replacement (Fx group)

These patients had primary osteoporosis according to the diagnostic criteria of the Japanese Society of Bone and Mineral Research and suffered from femoral neck fracture requiring femoral head replacement [21].

- (2) Patients with osteoarthritis of the hip (OA group)

These patients required hip replacement because of progressive osteoarthritis of the hip.

Patients were excluded from the study if they fitted any of the following exclusion criteria:

- 1) Patients with bacterial or viral (hepatitis B or C) infection
- 2) Patients with bone metastasis of cancer
- 3) Patients receiving warfarin therapy
- 4) Patients who had participated in a clinical trial of osteoporosis treatment within the previous 6 months
- 5) Patients who were judged to be inappropriate for this study by the attending physician (e.g., patients with rheumatoid arthritis)
- 6) Patients who had used vitamin K supplements within 1 month before giving informed consent
- 7) Patients using glucocorticoids (except for topical products) within 6 months before giving informed consent
- 8) Patients who had already undergone surgery on the contralateral proximal femur

Interview

After each patient gave written informed consent to the study, an interview was performed to obtain data on the history and lifestyle habits. The questions included the number of years after menopause, the number of deliveries, the performance of ovariectomy, smoking, alcohol consumption, complications, medical history, history of surgery, concomitant medications, coffee consumption, and consumption of natto (fermented soybeans).

Biochemistry tests and urinalysis

After admission to hospital and before surgery, 6 ml of blood and 10 ml of urine were collected from each patient. Serum was separated by centrifugation and was stored at -20°C until the following parameters were measured: intact osteocalcin (iOC; BGP IRMA “Mitsubishi,” Mitsubishi Chemical Medience Corporation, Tokyo, Japan), bone alkaline phosphatase (BAP; Osteolinks “BAP”, DS Pharma Biomedical Co., Ltd., Osaka, Japan), N-terminal telopeptide of type I procollagen (PINP; UniQ PINP RIA, Orion Diagnostica, Espoo, Finland), N-linked telopeptide of type I collagen (NTX; Osteomark NTx Serum, Inverness Medical Japan Co., Ltd., Tokyo), carboxylated OC (Gla-type Osteocalcin (Gla-OC) EIA Kit (Precoated), Takara Bio Inc., Shiga, Japan), and undercarboxylated OC (ucOC; Glu-OC Competitive EIA Kit, Takara Bio. Inc.). Urine levels of calcium (Ca), phosphate (P), and NTX (Osteomark, Inverness Medical Japan Co., Ltd.) were also measured and represented by the ratio to the level of creatinine (Cr). The central Laboratory of Mitsubishi Chemical Medience Corporation performed all of these measurements.

Spinal radiography

Conventional thoracic and lumbar spinal plain roentgenograms in lateral and anteroposterior projection were obtained from participants. Both lateral projections in the lateral decubitus position and A-P projections in the supine position were taken at a constant focus-film distance of 1.5 m. The severities of spinal degeneration including osteophyte formation, endplate sclerosis, disc space narrowing, and vertebral wedge deformity were assessed semi-quantitatively from Th4/5 to L4/5 disc level or from Th4 to L4 vertebrae by using the grading scale of Genant [22]. Then we assessed radiographical spinal disc degeneration using scoring system as previously reported [23–25]. Briefly, osteophyte formation at a given disc was graded 0–3°, endplate sclerosis at given vertebra was graded 0–2°, and disc space narrowing and vertebral wedge deformity were graded 0–1°, respectively. Then we defined sum of

each degree from Th4/5 to L4/5 disc level for osteophyte formation on anteroposterior radiographs as a score of osteophyte formation. We also defined sum of each degree from Th4 to L4 vertebra for endplate sclerosis and vertebral wedge deformity and that from Th4/5 to L4/5 disc level for disc space narrowing on lateral radiographs as a score of endplate sclerosis and disc space narrowing, respectively.

Bone mineral density measurement

Using dual energy X-ray absorptiometry (DXA; UOEH; Hologic-Delphi at Ken-ai-Kinen Hospital; Hologic-QDR 4500 at Shin-Kokura Hospital; Hologic QDR at Shin-Mizumaki Hospital; and Hologic QDR at Kitakyushu General Hospital), the lumbar bone mineral density (L2 to L4) and that of the proximal femur on the unaffected side were measured. The following parts of the proximal femur were evaluated: neck, trochanter, intertrochanteric region, total proximal femur, and Ward’s triangle. Differences of measured values between the different devices were corrected.

Harvesting of femoral heads

The femoral heads (including the neck) resected during surgery were cut into two coronal sections for measurement of bone tissue components and for bone histomorphometry. The sections for measurement of bone tissue components were stored at -20°C , after which each section was divided into cortical bone and cancellous bone components, and then crushed and pulverized. After dividing the femoral head in two using a hand saw, the cancellous bone was scraped away from the cortical bone using a chisel and spatula. The cancellous and cortical bone pieces were then, using nippers and a chisel, separately broken up into small fragments, which were washed in acetone and water to remove any adipose tissue. The resulting fragments were frozen and broken down using a micro dismembrator [CRYO-PRESS; Microtec Niton, Funabashi, Japan] to sample sizes able to pass through a 500- μm mesh, and again washed with acetone and water for delipidation. The resulting test samples were freeze-dried for 24 h and stored at -20°C . Measurements of OC, ucOC, and osteopontin (OPN) were performed using these test samples. Demineralization extraction was performed by reacting 1.5 mL 0.5 M of dimethyl sulfoxide (pH 7.4) containing 0.1 mL of 10% protease inhibitor (P8340, Sigma Co. Ltd), with a 20 mg initial sample at 4°C for 24 h. The resulting solution was centrifuged at 3,000 rpm for 5 min, and the supernatant then centrifuged at 10,000 rpm using an Ultrafree-MC filter [Millipore, MA, USA], and the supernatant used as the test sample. OC and OPN levels of the extracts were determined as for the blood samples, using the kits as described

above. Collagen derivatives were extracted by adding 2.0 mL of 6 mol/L HCl to a 40 mg initial sample.

Then the levels of OC (BGP IRMA “Mitsubishi,” Mitsubishi Chemical Medience Corporation), ucOC (Glu-OC Competitive EIA Kit, Takara Bio. Inc.), and OPN (Human Osteopontin Assay Kit-IBL, Immuno-Biomedical Laboratories Co., Ltd., Gunma, Japan) were measured in the bone tissue. Pyridinoline (PYD), deoxypyridinoline (DPD), and hydroxypyridine (HYP) were measured by high-performance liquid chromatography. All of the bone components were measured by the Central Laboratory of Mitsubishi Chemical Medience Corporation, while crushing and pulverizing of the bone samples was performed by the Central Research Laboratories of BML (Saitama, Japan).

The bone histomorphometry specimens were temporarily stored at 4°C in 4% paraformaldehyde immediately after sectioning. A 1-cm² of cancellous bone was resected from the center of each specimen. After immersion in Villaneuva staining solution, the bone squares were embedded in methyl methacrylate. Specimens were resected and embedded by staff at the University of Occupational and Environmental Health (Kitakyusyu, Japan).

Bone histomorphometry

For bone histomorphometry, specimens of bone were obtained from the center of the femoral head and were cut into sections 6 µm thick after being immersed in Villaneuva staining solution and embedded in methyl methacrylate. The sections were stained with Goldner stain before examination. The parameters that were measured or calculated for histomorphometry were the bone volume to total volume ratio (BV/TV; %), trabecular thickness (Tb.Th; µm), trabecular number (Tb.N; #/mm), trabecular separation (Tb.Sp; µm), osteoid volume to bone volume ratio (OV/BV; %), and osteocyte number to bone surface ratio (N.Ot/BS; #/mm). Calculations were performed by UOEH using a Histometry-RT-Camera (System Supply; Nagano, Japan). The abbreviations used are based on the nomenclature of the American Society for Bone and Mineral Research [26].

Parameters analyzed

Demographic characteristics analyzed included the age, time after menopause, height, weight, body mass index (BMI), and number of deliveries.

With regard to the past history and lifestyle habits, analysis included the performance of ovariectomy, smoking, alcohol consumption, complications, medical history, surgical history, concomitant medications, coffee consumption, and intake of natto (fermented soybeans).

The following bone metabolism markers were also assessed: serum iOC, serum BAP, serum PINP, serum NTX, urine NTX, serum Gla-OC, serum ucOC, ucOC/Gla-OC, Gla-OC/iOC, and ucOC/iOC.

Statistical analysis

Frequencies and percentages were calculated for discrete variables and ordinal categorical data. Summary statistics were calculated for continuous variables. Intergroup comparison of the OA and Fx groups were performed by using the χ^2 test for discrete variables, the *U* test for categorical data, and *t* test for continuous variables. For measured values from the same patient (continuous variables), comparisons were performed with the paired *t* test. The level of significance was set at $P \leq 0.05$ (two-sided), unless otherwise specified.

For investigation of factors affecting the presence/absence of fracture (OA group and Fx group), multivariate analysis was performed by multiple logistic regression analysis. This analysis employed the items that remained after screening by the following exclusion criteria:

1. Missing data (number of years after menopause, number of deliveries, presence/absence of ovariectomy, or presence/absence medical history)
2. Bone tissue components that might include cartilage (HYP and PYD)
3. Bone tissue components with a very low affinity for bone (ucOC)
4. Variables with a probability >0.15 according to univariate logistic regression analysis

The forward selection method, backward elimination method, and stepwise selection method were used to select variables. The level of significance for deciding whether a candidate variable would be included in the model was set at $P \leq 0.3$ (two-sided).

Since this study was exploratory, adjustment for multiplicity associated with verification of multiple hypotheses was not performed. All analyses were conducted with SAS software (release 9.1; SAS Institute Inc. Cary, NC, USA).

Results

Subjects

Forty-two patients gave written informed consent, and analysis of 39 patients was performed [18 in the OA group and 21 in the Fx group]. Three patients were excluded because radiography or DXA not performed ($n=2$) or because of a measurement date violation ($n=1$). None of the patients included in these analyses was taking inhibitors of bone resorption such as bisphosphonates or selective estrogen receptor modulators. In comparison with the OA

Table 1 Profile of the subjects

	Combined (<i>n</i> =39) Mean±SD	OA group (<i>n</i> =18) Mean±SD	Fx group (<i>n</i> =21) Mean±SD	<i>P</i> value (<i>t</i> test)
Age (years)	70.67±11.54	62.11±7.85	78.00±8.86	<0.001
Height (cm)	150.19±6.30	150.55±7.38	149.89±5.38	0.753
Weight (kg)	50.47±9.29	53.86±10.17	47.57±7.53	0.038
BMI	22.35±3.79	23.65±3.67	21.24±3.61	0.046
No. of years after menopause (year) ^a	19.41±12.39	13.50±9.79	27.29±11.30	0.002
No. of deliveries (number) ^b	2.24±1.26	2.17±1.20	2.31±1.35	0.743

Age and years after menopause were significantly greater, while weight and BMI were significant lower in the Fx group compared with the OA group

OA osteoarthritis, Fx fracture, BMI body mass index

^a Combined (*n*=28), OA group (*n*=16), Fx group (*n*=12)

^b Combined (*n*=34), OA group (*n*=18), Fx group (*n*=16)

group, the age was significantly greater, postmenopausal duration significantly longer, and body weight and BMI significantly lower in the FX group ($P<0.001$, $P=0.002$, $P=0.038$, and $P=0.046$, respectively: *t* test), but the height of the subjects and the number of deliveries showed no significant differences (Table 1). With respect to the past

history and lifestyle habits, significantly more patients in the Fx group had complications, a past medical history, and used concomitant medications ($P<0.001$, $P=0.005$, and $P=0.005$, respectively: χ^2 test). In the OA group, more patients had a high frequency of coffee consumption ($P=0.005$; *U* test, Table 2).

Table 2 Past history and lifestyle factors

	OA group (<i>n</i> =18)	Fx group (<i>n</i> =21)	<i>P</i> value
Ovariectomy	Yes	2	0
	No	16	17
Smoking	Yes	2	2
	No	16	19
Alcohol consumption	Never	12	19
	Occasionally	4	2
Complications	Regular	2	0
	Yes	7	19
Medical history	No	11	2
	Yes	6	15
Surgical history	No	12	4
	Yes	9	8
Concomitant medications	Yes	9	19
	No	9	2
Coffee consumption	Daily	7	3
	Occasionally	8	6
Intake of fermented soybeans	Rarely	3	12
	Regular	9	4
	About once weekly	5	10
	Never	4	7

The number of patients with complications, a medical history, or concomitant medications was significantly higher in Fx group. Coffee consumption was significantly greater in the OA group than the Fx group

^a χ^2 test

^b *U* test

Table 3 Serum and urine markers of bone metabolism

	OA group (n=18) Mean±SD	Fx group (n=21) Mean±SD	P value (t test)
Serum iOC (pg/ml)	4.89±2.52	4.94±2.53	0.952
Serum BAP (U/l)	28.47±10.03	26.47±9.76	0.533
Serum P1NP (pg/ml)	54.58±23.35	48.80±25.21	0.462
Serum NTX (nmolBCE/l)	17.58±4.71	18.91±4.75	0.388
Urine NTX (mmol BCE/mmol cr)	85.57±48.56	86.93±34.94	0.921
Serum Gla-OC (ng/ml)	18.46±7.32	19.00±6.54	0.809
Serum ucOC (ng/ml)	2.63±2.07	3.25±4.43	0.575
ucOC/Gla-OC	0.16±0.11	0.18±0.20	0.729
Gla-OC/iOC	5.18±3.70	4.72±2.23	0.647
ucOC/iOC	0.96±1.37	0.63±0.53	0.344
Ca/Cr	0.21±0.14	0.17±0.16	0.362
P/Cr	0.63±0.21	0.55±0.24	0.240

OA osteoarthritis, Fx fracture, iOC intact osteocalcin, BAP bone alkaline phosphatase, P1NP N-terminal telopeptide of type I procollagen, NTX N-linked telopeptide of type I collagen, Gla-OC carboxylated osteocalcin, ucOC undercarboxylated osteocalcin, Ca calcium, P phosphate, OC osteocalcin, Cr creatinine

Bone metabolism markers and bone minerals

The serum and urine markers of bone metabolism showed no significant differences between the two groups (Table 3). There were also no significant differences between the two groups with regard to serum ucOC/Gla-OC and Gla-OC/iOC or urine Ca/Cr and P/Cr.

Bone metabolism markers in cortical bone and cancellous bone

The OC level of cortical bone was significantly lower in the Fx group than in the OA group, but no significant difference was found for cancellous bone ($P<0.001$, $P=0.103$; t test, Table 4). ucOC was significantly lower in

Table 4 Components of cortical and cancellous bone

		OA group (n=18)		Fx group (n=21)		P value ^b
		Mean±SD	P value ^a (intragroup)	Mean±SD	P value ^a (intragroup)	
OC (ng/mg)	Cortical bone	277.45±113.55	<0.001	66.01±75.23	<0.001	<0.001
	Cancellous bone	346.61±116.32		282.80±122.21		0.103
ucOC (ng/mg)	Cortical bone	5.26±0.94	0.544	1.49±1.13	<0.001	<0.001
	Cancellous bone	5.10±1.09		4.37±1.12		0.044
OPN (ng/mg)	Cortical bone	138.51±45.01	0.865	15.32±6.60	<0.001	<0.001
	Cancellous bone	135.51±81.11		42.07±27.48		<0.001
PYD (pmol/mg)	Cortical bone	72.11±30.96	<0.001	525.68±168.88	<0.001	<0.001
	Cancellous bone	44.62±6.06		54.93±6.19		<0.001
DPD (pmol/mg)	Cortical bone	10.81±1.81	1.000	11.35±2.52	<0.001	0.442
	Cancellous bone	10.81±1.87		13.31±1.91		<0.001
HYP (nmol/mg)	Cortical bone	277.79±18.19	0.143	508.40±103.71	<0.001	<0.001
	Cancellous bone	270.84±18.55		257.78±19.10		0.037
Ca (µg/mg)	Cortical bone	229.34±23.82	0.028	129.31±79.38	<0.001	<0.001
	Cancellous bone	243.31±26.71		244.81±23.01		0.852

The OC level of cortical bone was significantly lower in the Fx group than in the OA group. ucOC and OPN were significantly lower and PYD was significantly higher in the Fx group than in the OA group for both cortical and cancellous bone. In the Fx group, the DPD level was significantly higher in cancellous bone than cortical bone. The HYP and Ca of cortical bone were significantly higher in the Fx group than the OA group. ucOC accounted for 1.67–2.22 percentage of total bone OC in both groups

OA osteoarthritis, Fx fracture, OC osteocalcin, ucOC undercarboxylated osteocalcin, OPN osteopontin, PYD pyridinoline, DPD deoxypyridinoline, HYP hydroxypyridine, Ca calcium

^a Paired t test

^b t test

Table 5 Collagen, non-collagen protein, and mineral composition ratios of cortical and cancellous bone

		OA group (n=18) Mean±SD	Fx group (n=21) Mean±SD	P value (t test)
Cortical bone	OC/Ca (×1,000)	1.21±0.49	0.51±0.26	<0.001
	OPN/Ca (×1,000)	0.61±0.20	0.15±0.09	<0.001
	DPD/Ca (×1,000)	0.05±0.01	0.11±0.05	<0.001
	OC/DPD (kg/mol)	25.75±9.10	5.59±5.15	<0.001
	OPN/DPD (g/mol)	13.10±4.12	1.39±0.58	<0.001
Cancellous bone	OC/Ca (×1,000)	1.45±0.52	1.16±0.48	0.083
	OPN/Ca (×1,000)	0.57±0.39	0.17±0.12	<0.001
	DPD/Ca (×1,000)	0.04±0.01	0.05±0.01	<0.001
	OC/DPD (kg/mol)	32.90±12.08	21.23±8.54	<0.001
	OPN/DPD (g/mol)	13.42±8.61	3.11±1.95	<0.001

The OC/Ca and OPN/Ca ratios of cortical bone were significantly higher in the OA group than the Fx group. However, the DPD/Ca ratio of cortical bone was significantly higher in the Fx group than in the OA group. The OPN/Ca and DPD/Ca ratios of cancellous bone were significantly lower in the Fx group than the OA group. The ratios of OC/DPD and OPN/DPD in cortical and cancellous bone were significantly lower in the Fx group than in the OA group

OA osteoarthritis, Fx Fracture, OC/Ca osteocalcin/calcium, OPN/Ca osteopontin/calcium, DPD/Ca deoxypyridinoline/calcium, OC/DPD osteocalcin/deoxypyridinoline, OPN/DPD osteopontin/deoxypyridinoline

the Fx group than in the OA group for both cortical bone and cancellous bone ($P<0.001$ and $P=0.044$, respectively: *t* test). OPN was also significantly lower in the Fx group than in the OA group for both cortical bone and cancellous bone (both $P<0.001$: *t* test). PYD was significantly higher in the Fx group than in the OA group for both cortical bone and cancellous bone (both $P<0.001$: *t* test). Although the DPD level of cortical bone showed no difference between the two groups, the Fx group had a significantly higher DPD level in cancellous bone ($P<0.001$: *t* test). The HYP level of cortical bone was significantly higher in the Fx group than the OA group ($P<0.001$: *t* test), but that level in cancellous bone were significantly higher in the OA group than in the FX group ($P=0.037$, *t* test).

The Ca level of cortical bone was also significantly higher in the Fx group ($P<0.001$; *t* test), but showed no significant difference between the two groups for cancellous bone.

ucOC accounted for 1.67–2.22 percentage of total bone OC in both groups, suggesting that ucOC shows a very low

affinity for bone. Therefore, it was judged to be unnecessary to investigate ucOC as a bone component and it was excluded from multivariate analysis. The differences of PYD and HYP levels between cortical bone and cancellous bone were much greater in the Fx group than in the OA group, probably due to loss of cartilage from the femoral head in the OA group, while cartilage was preserved in the Fx group. Therefore, it was judged that PYD and HYP values were influenced by the cartilage component and these variables were also excluded from multivariate analysis.

Non-collagen protein/Ca ratio and non-collagen protein/collagen ratio in cortical and cancellous bone

The ratio of OC to Ca (OC/Ca) in cortical bone and the ratio of OPN to Ca (OPN/Ca) in both cortical and cancellous bone were significantly lower in the Fx group than the OA group (all $P<0.001$: *t* test), while the ratio of DPD to Ca (DPD/Ca) was significantly higher in both cortical and cancellous bone ($P<0.001$ and $P=0.001$, respectively: *t* test). The ratios of OC and OPN to DPD in cortical bone and cancellous bone

Table 6 Results of bone density measurements

	Combined (n=39) Mean±SD	OA group (n=18) Mean±SD	Fx group (n=21) Mean±SD	P value (t test)
L2–4 (/cm ²)	0.790±0.113	0.821±0.106	0.763±0.114	0.112
Neck (/cm ²)	0.555±0.147	0.650±0.134	0.474±0.105	<0.001
Troch (/cm ²)	0.469±0.106	0.529±0.088	0.418±0.095	<0.001
Inter (/cm ²)	0.732±0.167	0.797±0.156	0.677±0.158	0.022
Total (/cm ²)	0.625±0.135	0.691±0.114	0.569±0.127	0.003
Ward's (/cm ²)	0.415±0.212	0.532±0.232	0.314±0.129	0.001

Table 7 Results of bone histomorphometry

	OA group (n=18) Mean±SD	Fx group (n=21) Mean±SD	P value (t test)
BV/TV (%)	24.22±0.23	14.70±4.52	<0.001
Tb.Th (µm)	225.58±118.07	161.47±50.86	0.043
Tb.N (/mm)	4.84±2.07	4.12±1.98	0.274
Tb.Sp (µm)	770.93±432.05	1,055.60±552.45	0.079
OV/BV (%)	20.15±16.04	17.51±11.70	0.568
N.Ot/BS (/mm)	5.63±3.42	7.71±5.97	0.185

BV/TV and Tb.Th were significantly lower in the Fx group than in the OA group

BV/TV bone volume/tissue volume, Tb.Th trabecular thickness, Tb.N trabecular number, Tb.Sp trabecular separation, OV/BV osteoid volume/bone volume, N.Ot/BS number of osteocyte/bone surface

were all significantly lower in the Fx group than the OA group ($P<0.001$, $P=0.001$, $P<0.001$, and $P<0.001$, respectively: *t* test; Table 5).

Radiographic findings of the thoracic and lumbar spine

More patients had deformities of the vertebral bodies ($P<0.001$: χ^2 test), but there was a higher number of vertebral deformities ($P=0.024$: *t* test) in the Fx group than in the OA group. When the thoracic and lumbar spine were compared, the Fx group had significantly more deformed lumbar vertebrae ($P=0.009$: *t* test). The presence/absence of narrowing of the intervertebral disks did not differ between the two groups, but there were significantly more narrow intervertebral disks in the Fx group than the OA group ($P=0.038$: *t* test). No significant differences were found for the other parameters (data not shown).

Bone mineral density measurements

Bone densities of the femoral neck were significantly lower in all sites in the FX group than in the OA group (Table 6).

Histomorphometry of cancellous bone in the femoral head

BV/TV and trabecular thickness were significantly lower in the Fx group than in the OA group ($P<0.001$ and $P=0.043$, respectively: *t* test). No significant differences were found for the other parameters (Table 7).

Results of multivariate analysis

Multiple logistic regression analysis was performed by using the presence/absence of fracture (OA group and Fx group) as the response variables and the analytical parameters as the explanatory variables.

Variables fitting the following criteria were excluded as candidate variables for the model:

1. Variables with missing data (postmenopausal duration, parity, oophorectomy or past history)
2. Variables discussed at the interim resession, conducted before all data was collated, for which multivariate analysis was considered unnecessary (bone ucOC, HYP, PYD)
3. Variables with a probability greater than 15% on univariate logistical regression analysis

As a result, we performed variable selection on the following variables using minimum redundancy, maximum relevance, and minimum redundancy maximum relevance selection. The two-tailed significance level for candidate variables included in the model was set at 30%.

Patient background factors

Age, body weight, BMI, intake of fermented soybeans, alcohol consumption, coffee consumption, complications

Bone constituents

Cortical bone: OC, Ca, OC/DPD, OC/Ca, OPN/Ca, DPD/Ca

Cancellous bone: OC, DPD, OPN, OC/DPD, OC/Ca, OPN/DPD, OPN/Ca, DPD/Ca

Table 8 Results of multivariate logistic regression analysis (N=39)

	Variable	Adjusted odds ratio	95% confidence interval (Wald)	
			Lower limit	Upper limit
Forward selection method	OC/DPD in cortical bone	0.493	0.079	3.086
	OPN/Ca in cortical bone	<0.001	<0.001	>999.999
Backward elimination method	OPN/Ca in cortical bone	<0.001	<0.001	0.001
Stepwise forward selection method				

OC/DPD osteocalcin/deoxypridinoline, OPN/Ca osteopontin/calcium

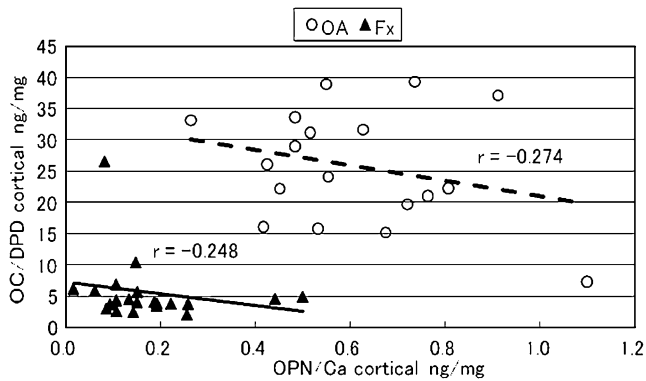


Fig. 1 Correlation between the OC/DPD ratio and OPN/Ca ratio of cortical bone in the two groups. Number of patients in each group was 11. OA osteoarthritis, Fx fracture, OC/DPD osteocalcin/deoxypyridinoline, OPN/Ca osteopontin/calcium

Bone density

L2–4 vertebrae, femoral neck, troch, inter, total, Ward's

Radiological evaluation

Number of narrowed intervertebral disks, mean osteophyte surface area (L1/2–4/5), presence of deformed vertebral bodies, number of deformed vertebral bodies, number of deformed thoracic vertebrae, Walter Reed Visual Assessment Scale for scoliosis

Bone metabolism measurements

Cancellous bone mass, cancellous bone width, bone gap, cancellous bone OPN/osteoid mass, cortical bone OPN/osteoid mass, cortical bone OC/osteoid mass

The results of variable selection are given below. The results of minimum redundancy selection and minimum redundancy maximum relevance selection were in agreement (Table 8).

As a result, the factors that significantly predicted fracture were OC/DPD and OPN/Ca in cortical bone by the forward selection method, as well as OPN/Ca in cortical bone by both the backward elimination and forward selection methods (Table 8).

However, since the model employing the forward selection method was unstable, the age distribution of OC/DPD and OPN/Ca was investigated. Because of the significant difference in age distribution ($P < 0.001$: t test), the same multivariate analysis was conducted for a subpopulation stratified by age in the two groups (22 subjects aged 59–80 years old).

When subpopulation analysis was performed, similar results were obtained as for the analysis of all 39 subjects.

Maximum relevance selection placed two variables, cortical bone OC/DPD and cortical bone OPN/Ca. We therefore produced a scatter diagram of cortical bone OC/DPD and cortical bone OPN/Ca (Fig. 1) and examined the relationship between these 2 variables.

The distributions of cortical bone OC/DPD and cortical bone OPN/Ca were separate and distinct for each group.

Discussion

In the present study, we investigated factors that influence the occurrence of fragility fractures in patients with osteoporotic femoral neck fractures and patients with osteoarthritis of the hip. Multiple logistic regression analysis identified OC/DPD and OPN/Ca of cortical bone as important predictors of the risk of fragility fracture, both of which have a similar predictive value.

Although bone mineral density of the lumbar spine tended to be lower, and femoral neck and intertrochanter bone mineral density were significantly lower in the FX group than in the OA group, the results of this study agree with previous reports indicating a relatively small contribution by bone mineral density to bone strength [17, 18]. On the other hand, decreases in either OC/DPD or OPN/Ca were significant predictive factors for fragility fracture, and low bone mineral density was not a predictive factor. Decreases in bone volume or bone formation are not observed in either osteocalcin gene or osteopontin gene deficit mice [27, 28].

Osteocalcin suppresses formation of calcium phosphate nuclei, and an osteocalcin deficit does not result in a reduction in calcification [29]. Decreased levels of fully carboxylated osteocalcin inhibit calcification crystal accumulation and cause random calcification of collagen fibers. This random calcification of collagen fibers can cause bone fragility irrespective of the mineral density [30] or early destruction of the collagen fibers [31]. These earlier reports agree with the findings of this study that decreased OC/DPD causes bone vulnerability independent of the bone mineral density. To maintain high quality bone, osteocalcin strictly regulates the formation of calcium phosphate nuclei, accumulation of calcified crystals, and the calcification of collagen fibers.

Osteopontin suppresses the growth of calcium phosphate crystals, and an osteocalcin deficit does not result in reduced calcification or bone formation [28, 29]. Although it is also produced by immature osteoblasts, 55 kDa osteopontin is synthesized mainly by mature osteoblasts prior to the precipitation of calcium phosphate crystals, and controls the growth of calcified crystals [32]. Low OPN/Ca conditions cause the accumulation of large calcified crystals, possibly reducing bone strength in spite of increased calcification [31].

Osteoblasts synthesize type 1 collagen from an early stage of differentiation [33], but most osteocalcin and osteopontin are produced by more mature osteoblasts [29].

Decreases in both the osteocalcin/type 1 collagen ratio and the osteopontin/calcium ratio indicate that patients with bone vulnerability suffer from a disturbance of osteoblast maturation. Promotion of osteoblast maturation is important in avoid fragility fracture in patients with osteoporosis, but no clinically applicable methods have so far been discovered. It is however possible that facilitation of osteocalcin carboxylation may increase osteocalcin uptake from the circulation and normalize abnormal calcification. It has been reported that reduced osteocalcin uptake correlates with the occurrence of fragility fractures [34]. It was also reported that vitamin K reversed warfarin-induced random calcification of collagen fibers in an animal study [30].

In a previous study of the relationship between decreased osteocalcin carboxylation and increased fracture risk [35], the serum level of undercarboxylated osteocalcin was an explanatory factor, but no significant difference was seen between groups in this parameter in the present study. This is likely because the capacity for osteocalcin synthesis cannot be assessed by measuring levels of carboxylation. The most important new finding of the present study is that carboxylation should be assessed with reference to the level of osteocalcin synthesis, rather than simply evaluating bone fragility from circulating levels of undercarboxylated osteocalcin. In other words, increased levels of undercarboxylated osteocalcin are a problem, as reported previously [34, 35], but only if there has been no alteration in the level of osteocalcin synthesis. If the ability to synthesize osteocalcin is reduced, we would naturally expect a decrease of undercarboxylated osteocalcin. In the present study, blood levels of undercarboxylated osteocalcin were similar in the FX and OA groups, despite the reduced osteocalcin synthesis capacity in the former group, suggesting a decrease in the extent of carboxylation. Although we have stated that impaired differentiation of osteoblasts was a crucial factor in fragility fractures, at present the only way we have to elucidate the status of osteoblasts is bone biopsy. Determination of bone uptake of aspartic acid, needed to synthesize osteopontin, may be a good predictor of bone vulnerability in combination with serum undercarboxylated osteocalcin levels.

This study has a number of limitations. Firstly, we used patients with end-stage osteoarthritis of the hip as the control group. The femoral heads of these patients contain osteosclerotic lesions and cannot be considered normal bone. However, this was an exploratory study, so we opted for using specimens that could be obtained during routine treatment without additional invasiveness. Another limitation is that many Japanese patients with osteoarthritis of the hip develop it secondarily to dysplasia of the acetabulum. The osteoarthritis group was therefore younger than the fracture group, necessitating statistical adjustment of the patient characteristics. Furthermore, when investigating

bone structure it is considered important to assess osteoid and osteocytes in cancellous bone, and we used data obtained by two-dimensional morphometry in the present study. Different results might have been obtained if we had performed microcomputed tomography analysis.

In conclusion, our assessment revealed that the OC/DPD and OPN/Ca ratios of cortical bone are important predictors of fragility fracture and a decrease of non-collagenous protein synthesis by osteoblasts and of osteocalcin uptake are possible causes of fragility fracture.

Conflicts of interest None.

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