Bone Turnover Markers and Risk Factors Associated with Osteoporosis and Decreased Bone Mass

Tomoko Shiga¹, Yuriko Moriyoshi¹, Hikaru Nagahara²

Abstract

Objective: The first aim of this study was to identify risk factors associated with osteoporosis and decreased bone mass in the general Japanese population. The second was to elucidate the relationship between bone mineral density and bone turnover markers.

Materials and Methods: We analyzed bone mineral density (BMD) and bone turnover markers - such as bone-specific alkaline phosphatase (BAP), urinary deoxypyridinoline (DPD) and N-terminal crosslinking telopeptide of type I collagen (NTX) - in 238 subjects who visited the Department of General Medicine, National Center for Global Health and Medicine from March 2005 through March 2007 for a Ningen Dock-style annual health check-up. Risk factors associated with osteoporosis and decreased bone mass were evaluated using multivariate logistic regression analysis, and the differences in BMD and bone turnover markers were examined between subjects with and without osteoporosis.

Results: Factors associated with osteoporosis were age, gender (female) and low BMI, and specifically in female subjects menopause, high serum level of total cholesterol and large alcohol intake \((p<0.05)\). BMD was strongly correlated with bone turnover markers. \((NTX: R= -0.44, p<0.0001; DPD: R= -0.42, p<0.0001)\).

Conclusion: Bone turnover markers were useful for estimating BMD. High serum level of total cholesterol and large alcohol intake were risk factors for osteoporosis.

Keywords Ningen Dock, osteoporosis, bone turnover marker

Osteoporosis, a result of abnormal mineral metabolism, is one of the most common disorders in elderly people, and bone fractures caused by osteoporosis are a major public health problem³. Patients with osteoporosis have no symptoms until they suffer a bone fracture, which will decrease their quality of life. Hence, prevention of osteoporosis is of great importance in maintaining the quality of life of elderly people² and reducing medical expenditure on the treatment of fractures³⁴. In this study, we evaluated several risk factors associated with osteoporosis in order to develop an approach towards preventing bone fractures.

A number of experimental and clinical studies have demonstrated that markers of bone metabolism can be used to investigate skeletal remodeling under normal and abnormal conditions. Biochemical markers associated with bone metabolism have been employed as non-invasive and comparatively inexpensive tools for the diagnostic work-up and management of metabolic bone disease, such as postmenopausal osteoporosis⁵. The markers presently used include osteoblast- and osteoclast-derived enzymes as well as structural peptides, precursors or fragments derived from the various compartments of the bone matrix⁶. These components are usually categorized into 2 types of markers; one is related to bone formation and the other represents bone resorption for clinical and didactic purposes⁵. In this study, we analyzed relationships between BMD and bone turnover markers to determine which markers are useful for predicting bone mass reduction.

BAP is a marker that represents bone formation because it is derived from osteoblasts. BAP is produced by osteoblasts at a relatively immature stage of differentiation and triggers calcification of bone tissue through the provision of phosphoric acid. BAP serum levels are not influenced by liver or kidney function⁷⁸. DPD and NTX are markers of bone resorption. Eighty five to 90% of bone matrix consists of type I collagen⁹⁻¹², in which DPD is incorporated abundantly. DPD is a cross-linker between type I collagen and telopeptides and is formed during extracellular maturation of fibrillar collagen and released during the dissociation.

¹ Department of General Medicine, National Center for Global Health and Medicine; ² Aoyama Hospital, Tokyo Women’s Medical University

Contact : Tomoko Shiga, Department of General Medicine, National Center for Global Health and Medicine, 1–21–1 Toyama Shinjuku-ku, Tokyo 162–8655, Japan. Tel: +81–3–3202–7181; Fax: +81–3–3202–8007; E-mail: toshiga@hosp.ncgm.go.jp
process of mature collagen. DPD is present at high concentration in mature bone collagen, but is absent from cartilage and skin and thus it is a specific marker for bone tissue. DPD in serum is normally excreted into the urine with as much as 40% in the free form because of its low molecular weight (429–591 Dalton). Owing to this, serum levels of DPD are extremely low and undetectable in normal individuals. DPD concentrations are therefore estimated in urine.

NTX, a peptide derived from bone type I collagen degraded during bone resorption, is released into the circulation and excreted into urine. Both urinary and serum levels of NTX are measurable, and the urinary level can be corrected according to the patient’s renal function.

Subjects and methods

Study population

Two hundred thirty-eight subjects (90 men aged 61.5 ± 13.1 years and 148 women aged 58.2 ± 12.6 years) who visited Department of General Medicine, National Center for Global Health and Medicine (Tokyo, Japan) from March 2005 through March 2007 for a Ningen Dock-style annual health check-up were analyzed. The individuals’ BMD and bone turnover markers, such as BAP, DPD, NTX, were examined. We excluded subjects who were being treated for osteoporosis and/or with steroidal medicines, and those with bone metastasis of malignancies as well as subjects who had undergone gynecological operations, those with renal dysfunction, hyperthyroidism, and infection with hepatitis B virus (HBV) and/or hepatitis C virus (HCV). Informed consent was obtained from all subjects.

Measurement of bone mineral density

BMD was measured at the L1–L4 level of the lumbar spine by dual energy X-ray absorptiometry (DXA) using a QDR-4500 bone densitometer (Hologic Inc. NY, USA). BMD was automatically calculated by dividing bone mineral content (BMC) (g) by bone area (cm²), expressing it in g/cm².

Definition of Osteoporosis and bone loss

In this study, osteoporosis and decreased bone mass were defined according to the Japanese diagnostic criteria; osteoporosis as BMD less than 70% of that of young adult mean (YAM) (age, 20–44 years) and decreased bone mass as 70% ≤ BMD < 80% of YAM. The normal range is 80% of YAM or higher.

Bone turnover markers

We adopted serum BAP as a marker of bone formation and DPD and NTX as markers of bone resorption. These and other bone turnover markers are influenced by gender, aging and menopause as well as circadian rhythm; their serum levels are high in the morning and low in the afternoon for the most part. The measurement of bone turnover markers and determination of normal limits in urine are conducted on fasting samples obtained in the morning and venous blood samples for measurement are also collected in the morning under fasting conditions.

Serum BAP levels were measured by chemiluminescent enzyme immunoassay (CLEIA) while urinary DPD and NTX levels were measured by enzyme immunoassay (EIA) using first or second void morning urine samples, and corrected for serum creatinine levels.

Definition of risk factors

The Ningen Dock-style annual health check-up includes the following: physical characteristics (height, body weight and waist circumference), complete blood count, blood biochemistry, urinalysis, electrocardiogram, abdominal ultrasonography, chest radiography, barium meal examination of the upper gastrointestinal tract or upper gastrointestinal endoscopy, visual acuity test, tonometry, fundus examination (retinal photography) and hearing ability. Medical histories and information on alcohol consumption- and tobacco consumption-related lifestyle factors are obtained in a personal interview with a physician.

We defined the potential risk factors as follows: alcohol overuse (over 20 g of ethanol per day); smoking (Brinkman Index (B.I.) > 200, number of cigarettes smoked per day multiplied by number of years of smoking); hypertension (systolic blood pressure ≥ 130 mmHg, diastolic blood pressure ≥ 85 mmHg), and/or presence of antihypertensive drug treatment; low BMI (body mass index (BMI) < 18.5 kg/m²), calculated using weight in kilograms divided by the square of the subject’s height in meters); high levels of total cholesterol (total cholesterol ≥ 220 mg/dL, and/or presence of drug treatment for lipid abnormalities); high levels of LDL cholesterol (LDL cholesterol ≥ 140 mg/dL and/or presence of drug treatment for hyperlipidemia); low levels of HDL cholesterol (HDL cholesterol < 40 mg/dL and/or presence of drug treatment for hyperlipidemia); hypertriglyceridemia (triglyceride ≥ 150 mg/dL and/or presence of drug treatment for hypertriglyceridemia); abnormal lipid metabolism (total cholesterol ≥ 220 mg/dL, low density lipoprotein cholesterol (LDL-C) ≥ 140 mg/dL, high density lipoprotein cholesterol (HDL-C) < 40 mg/dL, triglyceride (TG) ≥ 150 mg/dL, and/or presence of drug treatment); glucose intolerance (fasting plasma glucose (FPG) ≥ 110 mg/dL, and/or presence of drug treatment for diabetes mellitus (DM)); hyperuricemia (uric acid > 7 mg/dL, and/or presence of drug treatment for hyperuricemia).

Statistical analysis

Statistical analysis was performed using computer software (IBM SPSS Statistics, version 19.0 IBM corporation, New York, USA). Continuous variables were expressed as means ± SD for each subject group. Statis-
tical differences were determined by the two-sided Student’s t-test (for equal variance cases) or Welch’s t-test (for non-equal variance cases). Non-normally distributed variables were compared by the Mann-Whitney U test. Variables given as proportions were compared using the chi-square test. Relationships between osteoporosis and risk factors were examined by multivariate logistic regression analysis. To analyze relationships between BMD and bone turnover markers, we created scattering profiles by separately plotting BMD data against those for individual bone turnover markers (BAP, DPD, NTX). A p-value < 0.05 was taken to be statistically significant.

This study was approved by the Ethics Committee of National Center for Global Health and Medicine (Approval No. NCGM-G-001188-00).

Results
Prevalence of osteoporosis and decreased bone mass
Of the 238 subjects, osteoporosis or decreased bone mass was detected in 35 (14.7%) and 30 (12.6%), respectively. The age- and sex-specific prevalences of osteoporosis and decreased bone mass are listed in Table 1. An increasing prevalence of osteoporosis with age was evident in female subjects (p<0.05), but not in male subjects.

Clinical characteristics and risk factors associated with osteoporosis and decreased bone mass
We first investigated the clinical characteristics and risk factors associated with osteoporosis or decreased bone mass. Table 2 shows there were more females than males with osteoporosis or decreased bone mass. Also, age and total cholesterol and HDL cholesterol levels were significantly higher in these subjects than in normal subjects. In contrast, waist circumference was significantly smaller and BMI and triglyceride, FPG and uric acid levels significantly lower in the former than the latter.

To investigate which risk factors were significantly related to osteoporosis and decreased bone mass, we performed contingency table analysis between the subjects with and without osteoporosis or decreased bone mass using multivariate logistic regression analysis (Table 3). Female gender (OR=4.45, p=0.005) and low BMI (OR=13.58, p<0.0001) and age (OR=1.09, p<0.0001) were statistically significant risk factors for osteoporosis and decreased bone mass.

Next we investigated risk factors specific for females. Table 4 shows the clinical characteristics of subjects

Table 1. Prevalence of Decreased Bone Mass and Osteoporosis

<table>
<thead>
<tr>
<th>Age</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Decreased bone mass</td>
</tr>
<tr>
<td>&lt;50</td>
<td>15</td>
<td>83.3%</td>
</tr>
<tr>
<td>50–59</td>
<td>15</td>
<td>83.3%</td>
</tr>
<tr>
<td>60–69</td>
<td>26</td>
<td>89.7%</td>
</tr>
<tr>
<td>70+</td>
<td>24</td>
<td>96.0%</td>
</tr>
</tbody>
</table>

Table 2. Clinical Characteristics and Laboratory Data of Study Subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Subjects with osteoporosis and decreased bone mass</th>
<th>Subjects without osteoporosis and decreased bone mass</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>63.29 ± 10.31 (65)</td>
<td>58.01 ± 13.46 (173)</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>(10/55)</td>
<td>(80/93)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>118.80 ± 14.61 (65)</td>
<td>120.64 ± 18.23 (173)</td>
<td>0.373</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>75.22 ± 10.12 (65)</td>
<td>76.95 ± 10.92 (173)</td>
<td>0.265</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>223.65 ± 45.80 (65)</td>
<td>209.90 ± 38.74 (173)</td>
<td>0.021</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>130.69 ± 38.65 (65)</td>
<td>132.33 ± 110.82 (173)</td>
<td>0.907</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>70.88 ± 16.35 (65)</td>
<td>61.79 ± 15.24 (173)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>107.69 ± 51.90 (65)</td>
<td>131.89 ± 88.57 (173)</td>
<td>0.01</td>
</tr>
<tr>
<td>FPG (mg/dL)</td>
<td>94.08 ± 9.32 (65)</td>
<td>102.62 ± 25.20 (173)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>4.90 ± 0.98 (65)</td>
<td>5.40 ± 1.51 (173)</td>
<td>0.004</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>23.83 ± 11.83 (65)</td>
<td>23.47 ± 7.99 (173)</td>
<td>0.79</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>20.91 ± 9.06 (65)</td>
<td>23.14 ± 12.13 (173)</td>
<td>0.127</td>
</tr>
<tr>
<td>γ-GTP (U/L)</td>
<td>39.66 ± 109.15 (65)</td>
<td>42.64 ± 62.28 (173)</td>
<td>0.793</td>
</tr>
<tr>
<td>hsCRP</td>
<td>0.69 ± 0.09 (65)</td>
<td>0.12 ± 0.31 (173)</td>
<td>0.173</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>21.11 ± 3.05 (65)</td>
<td>23.13 ± 3.24 (173)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>80.41 ± 9.01 (65)</td>
<td>84.56 ± 9.55 (173)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Results are shown as mean ± standard deviation (SD).

Ningen Dock International  Vol. 1 No. 1 2013  3 (3)
with and without osteoporosis. Only the proportion of post-menopausal women was significantly higher in subjects with osteoporosis than in those without it.

We then performed another contingency table analysis between subjects with and without osteoporosis using multivariate logistic regression analysis with osteoporosis as a dependent variable and the following 15 explanatory variables: menopause, low BMI, hypertension, high level of total cholesterol, high level of LDL cholesterol, low level of HDL cholesterol, hypertriglyceridemia, glucose intolerance, hyperuricemia, AST > 33 U/L, ALT > 42 U/L in males or ALT > 27 U/L in females, hsCRP > 0.145, alcohol overuse and past history of bone fracture. Menopause (OR=6.89, \( p=0.016 \)), high level of total cholesterol (OR=5.04, \( p=0.028 \)) and alcohol overuse (OR=5.04, \( p=0.041 \)) were statistically significant risk factors for osteoporosis among female subjects (Table 5).

### Relationships between bone mineral density and bone turnover markers

We assessed relationships between BMD and bone turnover markers (BAP, DPD, NTX) among both male and female subjects. We found that BMD was inversely correlated with NTX (\( R=-0.44, p<0.0001 \)) and DPD
(R = -0.42, p < 0.0001) and negatively correlated with BAP (R = -0.30, p < 0.0001) (Fig. 1).

Discussion

Osteoporosis is a disease characterized by low mineral density and an altered bone microstructure, which cause bone fragility. It is generally classified as the primary or secondary type. Risk factors conventionally identified for osteoporosis are advancing age, smoking, alcohol intake, low body weight, physical inactivity, low calcium intake, low vitamin D and estrogen status.

Osteoporosis most commonly affects postmenopausal women, increasing the risk of bone fractures. According to a study in the United States (U.S.), during the period 2005–2006, 4.5 million women aged >50 years (equal to 10% population of >50 years) had osteoporosis, and another 22.7 million (49%) had osteopenia based on femur neck bone mineral density (BMD) tests. There were 0.8 million men aged >50 years (0.3%) with osteoporosis, and another 11.8 million (30%) had osteopenia. In Japan, 30% of women in their seventies suffer from osteoporosis.

In the present study, the prevalences of osteoporosis and decreased bone mass also increased with age in female, but not in male subjects (Table 1). Hence, menopause is a risk factor for osteoporosis with a mechanism...
involving a lack of estrogen. However, the exact mechanism of the action of estrogen on bone remains unclear. Estrogen may stimulate synthesis of insulin-like growth factor I (IGF-I) and transforming growth factor-β (TGF-β) in osteoblasts but inhibit production of interleukin 1 (IL-1) and tumor necrosis factor-α (TNF-α) in monocytes. Thus in response to estrogen deficiency there could be great changes in the levels of these cytokines and this might account for the more rapid bone loss in post-menopausal women.25,26

We found that BMI was another risk factor for osteoporosis and decreased bone mass. A low BMI was correlated with osteoporosis and decreased bone mass with a high OR and low p-value (OR=13.58, p<0.0001, Table 3) among male and female subjects. The association between a low body weight and osteoporosis has usually been explained on the basis of small constitutional size in women, i.e. it is assumed that small women have small bones and also less reserve bone mass, which may serve to postpone the onset of osteoporosis27. Body weight is considered an important predictor of bone mineral density, and it has been observed that a low body weight raises the relative risk of fracture28. Body weight acts as a stimulus for bone formation and helps sustain bone mass.

We also observed that hypercholesterolemia and alcohol overuse were risk factors for osteoporosis in women (Table 5). Osteoporosis has been shown to be strongly correlated with arteriosclerotic vascular diseases, and it has been suggested that hyperlipidemia is a common pathogenic factor for arteriosclerosis. In this regard, in vitro studies have shown that LDL oxidation products had the ability to inhibit osteoblast differentiation and promote adipocyte differentiation29,30, suggesting that these products could be harmful to both the vessel wall and bone structure. In vivo studies have found that hyperlipidemia reduced bone density via increased osteoclastic bone resorption in mice31,32. However, little is known about a relationship between osteoporosis and hyperlipidemia from clinical studies. Some researchers have reported that hyperlipidemia reduced bone mineral density33–35 while others found no relationship between serum lipid levels and bone mineral density36.

Alcohol has definite direct effects on bone and mineral metabolism by suppressing osteoblast function, leading to diminished bone formation and defective mineralization37. Heavy drinkers may have poor nutrition with respect to calcium, vitamin D (due to liver dysfunction), or protein, causing bone loss38. In contrast, the effects of moderate alcohol intake are not deleterious to skeletal health. There is a threshold effect: no increase in osteoporotic or hip fracture risk was found in individuals who took 2 units (16g of ethanol) or less per day of alcohol, whereas a significant risk was noted if people exceeded this threshold39. In the present study, subjects with alcohol overuse were at high risk for osteoporosis (Table 5).

Urinary NTX levels are useful for determining whether individuals have normal or low bone density at the hip40 and we observed a statistically significant relationship between bone mineral density and bone turnover markers. (NTX : R=-0.44, p<0.0001, DPD: R=-0.42, p<0.0001). Thus bone turnover markers would be useful for estimating bone loss and determining the necessity of osteoporosis treatment. Yoshimura et al. also reported that bone resorption markers, especially DPD, were useful in predicting the risk of osteoporosis and vertebral fractures41.

Summing up, risk factors for osteoporosis were age, gender (female) and low BMI, and specifically in female subjects menopause, high serum level of total cholesterol and alcohol overuse. Bone turnover markers were strongly associated with BMD and would therefore be useful for estimating bone loss and selecting the optimal medication for osteoporosis.

The authors state that they have no Conflict of Interest (COI).

References


(Received June 11, 2013; Accepted August 9, 2013)