Factor Analysis of Biochemical Markers Associated with Bone Mineral Density in Adults

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Abstract. [Purpose] The aim of this study was to find biochemical markers related to low bone mineral density in Korean adults. [Subjects and Methods] From August 1 to September 15, 2013, subjects receiving medical checkups were classified as lumbar spine bone normal, osteopenic, or osteoporotic using a bone mineral densitometer. Next, age, body mass index, and biochemical parameter differences were compared among the three groups. [Results] The results revealed that, the relevant factors were maximum blood pressure, minimum blood pressure, bone mineral density, total bilirubin, alkaline phosphatase (ALP), fasting blood glucose, iron, neutrophils, monocytes, and eosinophils. The bone mineral density of patients with osteoporosis was 0.763 times lower than that of normal subjects. The total bilirubin level of patients with osteoporosis was 0.45 times lower than that of normal subjects. The alkaline phosphatase level of patients with osteopenia was 1.059 times higher than that of normal subjects, and that in patients with osteoporosis was 1.088 times higher than that in normal subjects. The fasting blood glucose level of patients with osteoporosis was 0.963 times lower than that of normal subjects. The iron level of patients with osteoporosis was 0.986 times lower than that of normal subjects. [Conclusion] In conclusion, osteoporosis is a representative disease in elderly women due to aging and menopause, and more active interest should be taken for prevention and treatment.

Key words: Osteopenia, Osteoporosis, Bone mineral density

INTRODUCTION

The aging of modern society due to cultural improvements and the development of medical technology, has resulted in an increase in age-related disease, which in turn has resulted in serious medical cost problems. In particular, osteoporosis is a representative age-related disease that is impacting society in terms of socioeconomics as well as quality of life in the elderly1–3). Osteoporosis is the most common metabolic bone disease in the elderly, and the World Health Organization (WHO) reported that susceptibility to fracture increases with decreased bone mass4–6). The US National Institutes of Health reported that osteoporosis is characterized as an increased fracture risk due to decreased bone strength in their recent NIH Consensus Statement and highlighted the relative importance of bone strength compared with bone mass7). In the USA, 8 million women are assumed to have osteoporosis, and 22 million people are assumed to have osteopenia. In the Republic of Korea, the prevalence of osteoporosis after menopause is 10%, and that of osteopenia is ~30%8–10). In addition, fractures of the spine, hip, and wrist are common in the case of severe osteoporosis. In particular, treatment and recovery from fractures are difficult in the elderly; thus, it is important to diagnose and treat osteoporosis early11). Weight is as an important factor in determination of bone mineral density (BMD) and the frequency of fractures12, 13). Other factors affecting BMD include deficient calcium intake, amount of exercise, hyperthyroidism affecting bone metabolism, hyperparathyroidism, and endocrine and metabolic diseases such as diabetes and chronic renal failure14, 15). However, studies on biochemical bone metabolism factors are lacking. Osteoporosis is usually diagnosed using BMD measurements. The quantitative measurement methods are radiographic absorptiometry (RA), dual energy X-ray absorptiometry (DEXA), quantitative computed tomography (QCT)/peripheral QCT (pQCT), quantitative ultrasound, and quantitative magnetic resonance16). Among the many methods, DEXA is mainly used for BMD measurement and is the most sensitive and appropriate standard method for osteoporosis assessments17, 18). In this study, BMD was...
measured using DEXA and biochemical markers associated with BMD in adult males and females were confirmed.

**SUBJECTS AND METHODS**

In this study, subjects receiving physical checkups were studied for an association between a physical examination, blood results, and BMD. From August 1 to September 15, 2013, 100 subjects were selected from among those who underwent a physical checkup including BMD measurement and a blood test and who were ≥ 50 years or menopausal women who had osteoporosis, osteopenia, or were normal as determined by their T-score. The study included 20 men and 80 women. Subjects with diabetes were excluded. The mean age of the men was 56.72±6.06 years, and their mean height was 168.97±6.02 cm. The mean age of the women was 58.21±5.96 years, and mean height was 154.31±5.37 cm. All participants signed a written informed consent form approved by the Institutional Review Board at Soonchunhyang University Hospital. The images were acquired using existing data.

BMD was measured using DXA (Lunar Bravo, Lunar Prodigy, or Lunar Advance; GE Healthcare Technologies, San Francisco, CA, USA) of the lumbar spine: L1–L4 were major markers of BMD. To improve test precision, the mean BMD for the 1st lumbar vertebra to the 4th lumbar vertebra (L1–L4) was used, and the left femur test was excluded due to low accuracy. According to the International Society for Clinical Densitometry (ISCD) classification, if the lumbar spine T-score was > −1.0, subjects were classified as normal; if it was −1.0 to −2.4, subjects had osteopenia, if it was < −2.5, subjects had osteoporosis. In addition to the physical examination, body weight and height were measured using an automatic height and weight scale. Body mass index (BMI) was calculated as weight (kg)/height squared (m$^2$). Biochemical parameters were measured after at least a 12 h fast through blood testing and urinalysis. Thirty-five items were collected as study variables, including maximum blood pressure, minimum blood pressure; minimum blood pressure; albumin, globulin, and total protein levels, albumin/globulin ratio (AG ratio); aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), uric acid, lactate dehydrogenase, cholesterol, triglyceride, fasting blood glucose, amylase, urea nitrogen, creatinine, and total bilirubin levels; blood urea nitrogen / creatinine ratio (BUN/Cr ratio); iron, cell, and hemoglobin levels; hematocrit; mean corpuscular volume; mean corpuscular he-

| Table 1. Values of the three groups of normal subjects and patients with osteopenia and osteoporosis |
|------------------------------|---------------|------------------------------|---------------|
| Variable                  | Mean          | Variable                  | Mean          |
|------------------------------|---------------|------------------------------|---------------|
| BMI (kg/m$^2$)              |               | Uric acid (mg/dl)         |               |
| Normal                      | 23.89±2.53    | Osteopenia                 | 4.96±1.08     |
| Osteopenia                  | 23.01±2.84    | Osteoporosis                | 4.69±1.37     |
| Osteoporosis                | 22.12±2.29    |                             |               |
| Total protein (g/dl)        |               | LDH (u/L)$^6$              |               |
| Normal                      | 7.09±0.30     | Osteopenia                 | 188.98±30.64  |
| Osteopenia                  | 7.22±0.38     | Osteoporosis                | 198.05±37.56  |
| Osteoporosis                | 7.26±0.31     |                             |               |
| Albumin (g/dl)              |               | Cholesterol (mg/dl)        |               |
| Normal                      | 4.21±0.18     | Osteopenia                 | 197.70±38.14  |
| Osteopenia                  | 4.20±0.22     | Osteoporosis                | 201.35±31.50  |
| Osteoporosis                | 4.31±0.27     |                             |               |
| Globulin (g/dl)             |               | Triglyceride (mg/dl)       |               |
| Normal                      | 2.89±0.23     | Osteopenia                 | 114.74±45.65  |
| Osteopenia                  | 3.02±0.36     | Osteoporosis                | 118.68±49.82  |
| Osteoporosis                | 2.95±0.21     |                             |               |
| Albumin/globulin ratio (%)  |               | FPG (mg/dl)$^7$            |               |
| Normal                      | 1.48±0.13     | Osteopenia                 | 99.88±19.18   |
| Osteopenia                  | 1.41±0.19     | Osteoporosis                | 92.62±14.71   |
| Osteoporosis                | 1.77±1.90     |                             |               |
| Total bilirubin (mg/dl)     |               | Amylase (mg/dl)            |               |
| Normal                      | 1.03±0.31     | Osteopenia                 | 64.19±23.81   |
| Osteopenia                  | 0.88±0.40     | Osteoporosis                | 66.84±25.19   |
| Osteoporosis                | 0.73±0.22     |                             |               |
| AST (u/L)$^2$               |               | Blood urea nitrogen (mg/dl) |               |
| Normal                      | 22.28±6.29    | Osteopenia                 | 14.67±3.92    |
| Osteopenia                  | 22.95±10.06   | Osteoporosis                | 13.84±3.12    |
| Osteoporosis                | 21.51±5.63    |                             |               |
| ALT (u/L)$^3$               |               | Creatinine (mg/dl)         |               |
| Normal                      | 24.33±8.79    | Osteopenia                 | 0.85±0.15     |
| Osteopenia                  | 22.26±11.50   | Osteoporosis                | 0.81±0.14     |
| Osteoporosis                | 19.43±7.16    |                             |               |
| GGT (u/L)$^4$               |               | BUN/Cr ratio (%)$^8$       |               |
| Normal                      | 36.56±29.14   | Osteopenia                 | 18.26±4.84    |
| Osteopenia                  | 39.95±39.74   | Osteoporosis                | 18.60±5.84    |
| Osteoporosis                | 23.65±14.48   |                             | 18.34±5.58    |
moglobin volume; red blood cell size distribution; platelet count; mean platelet volume; platelet distribution factor; white blood cell count; and neutrophil, lymphocyte, monocyte, and eosinophil percentages.

Lumbar spine BMD was classified into three groups, normal, osteopenia, and osteoporosis, and the characteristics were compared. First, differences in age, BMI, and biochemical markers were tested among the three groups by ANOVA. After the univariate analysis, factors showing significant relevance to BMD were evaluated by multivariate logistic regression analysis. The statistical analysis was performed using the SPSS ver. 18.0 software (SPSS Inc., Chicago, IL, USA). A p < 0.05 was considered significant.

RESULTS

BMI was 22.12 ± 2.28 kg/m² in patients with osteoporosis and 23.89 ± 2.52 kg/m² in the normal group (p<0.05). The total bilirubin level was 0.73±0.22 mg/dl in patients with osteoporosis and 1.03±0.31 mg/dl in the normal group. (p<0.05). The GGT level of patients with osteoporosis was 23.64±14.47 u/L and was significantly lower (p<0.05) than that of the normal group. No differences were observed in the remaining blood parameters (Table 1). Next, factors showing significant relevance to BMD were evaluated in a univariate analysis. Factors showing relevance were/ maximum blood pressure; minimum blood pressure; BMI; ALP, fasting blood glucose, iron, and total bilirubin levels; and neutrophil, monocyte, and eosinophil counts (p<0.05). The maximum blood pressure in patients with osteopenia was 1.167 times higher than that in patients with osteoporosis and 1.203 times higher than that in normal subjects. The minimum blood pressure in patients with osteopenia was 0.812 times lower and that in patients with osteoporosis and 0.834 times lower than that in normal subjects. The BMI of patients with osteoporosis was 0.763 times lower than that of normal subjects. The total bilirubin level in patients with osteoporosis was 0.45 times lower than that of normal subjects. The ALP level of patients with osteopenia was 1.059 times higher than that of normal subjects, and ALP of patients with osteoporosis was 1.088 times higher than that of

| Variable | Mean (Normal) | Mean (Osteopenia) | Mean (Osteoporosis) |
|----------|---------------|-------------------|---------------------|
| ALP (u/L) | 51.06±14.22 | 75.67±19.80 | 88.38±24.52 |
| Red blood cell count (10^9 /um) | | | |
| Normal | 4.57±0.36 | 4.48±0.35 | 4.46±0.41 |
| Hemoglobin (g/dl) | 14.10±1.01 | 13.89±1.20 | 13.56±1.31 |
| Hematocrit (%) | 41.12±2.62 | | |
| MCV (fl) | 40.69±3.00 | 40.25±3.36 | 40.89±4.02 |
| MCH (ug) | 31.00±1.47 | 30.39±1.42 | 30.91±1.11 |
| MCHC (%) | 34.29±0.59 | 34.11±0.81 | 33.65±0.70 |
| RDW (%) | 12.75±0.63 | 12.90±0.54 | 12.85±0.59 |
| Platelet count (10^11/um) | 231.83±40.43 | 238.86±51.16 | 239.68±48.67 |

1. Body mass index, 2. aspartate aminotransferase, 3. alanine aminotransferase, 4. gamma-glutamyl transferase, 5. alkaline phosphatase, 6. lactate dehydrogenase, 7. fasting plasma glucose, 8. blood urea nitrogen / creatinine ratio, 9. mean corpuscular volume, 10. mean corpuscular hemoglobin, 11. mean corpuscular hemoglobin concentration, 12. red cell distribution width, 13. mean Platelet Volume, 14. platelet distribution width

Table 1. Continued

(p<0.05). The GGT level of patients with osteoporosis was 23.64±14.47 u/L and was significantly lower (p<0.05) than that of the normal group. No differences were observed in the remaining blood parameters (Table 1). Next, factors showing significant relevance to BMD were evaluated in a univariate analysis. Factors showing relevance were/ maximum blood pressure; minimum blood pressure; BMI; ALP, fasting blood glucose, iron, and total bilirubin levels; and neutrophil, monocyte, and eosinophil counts (p<0.05). The maximum blood pressure in patients with osteopenia was 1.167 times higher than that in patients with osteoporosis and 1.203 times higher than that in normal subjects. The minimum blood pressure in patients with osteopenia was 0.812 times lower and that in patients with osteoporosis and 0.834 times lower than that in normal subjects. The BMI of patients with osteoporosis was 0.763 times lower than that of normal subjects. The total bilirubin level in patients with osteoporosis was 0.45 times lower than that of normal subjects. The ALP level of patients with osteopenia was 1.059 times higher than that of normal subjects, and ALP of patients with osteoporosis was 1.088 times higher than that of
The fasting blood glucose level in patients with osteoporosis was 0.963 times lower than that in normal subjects. The iron level in patients with osteoporosis was 0.986 times lower than that in normal subjects. The neutrophil count in patients with osteoporosis was 1.072 times higher than that in normal subjects. The monocyte count in patients with osteoporosis was 0.697 times lower than that in normal subjects. The eosinophil count in patients with osteoporosis was 0.723 times lower than that in normal subjects (p<0.05) (Table 2).

**DISCUSSION**

Osteoporosis is defined as “a musculoskeletal disease with an increased risk of fractures due to bone strength weakness” by the NIH19 and is mainly expressed by BMD19. The T-score is calculated as: (measured value in patient – mean value in young population)/standard deviation and is compared to the BMD in the young population showing the highest bone mass to reveal relative risks for fracture19. Osteoporosis is a disease of decreasing bone mass due to decreased bone formation and bone destruction resulting from various localized factors and systemic factors that modulate bone cell function. In addition, many hormones, cytokines, and genes are involved in this mechanism20. Unlike the above factors, studies on biochemical bone metabolic markers are lacking. In the present study, BMD was measured by DEXA and biochemical markers were checked in adult male and female subjects. The factors that showed relevance were BMI; maximum blood pressure; and

| Division         | OR (95% CI) | Minimum | Maximum |
|------------------|-------------|---------|---------|
| Osteopenia       | 1           | 1       | 1       |
| BMI (kg/m)       | 0.876       | 0.707   | 1.087   |
| Osteoporosis     | 1           | 1       | 1       |
| BMI (kg/m)       | 0.763       | 0.606   | 0.962   |
| Osteopenia       | 1           | 1       | 1       |
| Total bilirubin (mg/dl) | 0.346     | 0.078   | 1.539   |
| Osteoporosis     | 1           | 1       | 1       |
| Total bilirubin (mg/dl) | 0.045     | 0.006   | 0.358   |
| Osteopenia       | 1           | 1       | 1       |
| ALP              | 1.059       | 1.016   | 1.105   |
| Osteoporosis     | 1           | 1       | 1       |
| ALP              | 1.088       | 1.041   | 1.137   |
| Osteopenia       | 1           | 1       | 1       |
| FPG (mg/dl)      | 0.997       | 0.967   | 1.028   |
| Osteoporosis     | 1           | 1       | 1       |
| FPG (mg/dl)      | 0.963       | 0.924   | 1.003   |
| Osteopenia       | 1           | 1       | 1       |
| Fe               | 0.998       | 0.987   | 1.009   |
| Osteoporosis     | 1           | 1       | 1       |
| Fe               | 0.986       | 0.972   | 1       |
| Osteopenia       | 1           | 1       | 1       |
| Neutrophils (%)  | 1.055       | 0.989   | 1.125   |
| Osteoporosis     | 1           | 1       | 1       |
| Neutrophils (%)  | 1.072       | 1.003   | 1.147   |
| Osteopenia       | 1           | 1       | 1       |
| Monocytes (%)    | 0.777       | 0.562   | 1.073   |
| Osteoporosis     | 1           | 1       | 1       |
| Monocytes (%)    | 0.697       | 0.494   | 0.984   |
| Osteopenia       | 1           | 1       | 1       |
| Eosinophils (%)  | 0.881       | 0.698   | 1.113   |
| Osteoporosis     | 1           | 1       | 1       |
| Eosinophils (%)  | 0.723       | 0.536   | 0.974   |

1Body mass index, 2aspartate aminotransferase, 3alanine aminotransferase, 4gamma-glutamyl transferase, 5alkaline phosphatase, 6lactate dehydrogenase, 7fasting plasma glucose, 8blood urea nitrogen / creatinine ratio, 9mean corpuscular volume, 10mean corpuscular hemoglobin, 11mean corpuscular hemoglobin concentration, 12red cell distribution width, 13mean Platelet Volume, 14platelet distribution width
minimum blood pressure; ALP, fasting blood glucose, iron, and total bilirubin levels; and neutrophil, monocyte, and eosinophil counts. Patients with osteoporosis had a 0.763 times lower BMI than that of normal subjects. Weight and bone mass are closely related, so bone mass is low and bone loss increases in postmenopausal women with a low BMI, but BMD tends to be high in obese women. As weight increases, the load on the muscles increases and more mechanical stress is loaded, so bone mass is maintained. Blood bilirubin is negatively associated with smoking, low-density lipoprotein cholesterol, diabetes, and obesity. Maugeri et al. reported that the BMD in a diabetic group was lower than that in a nondiabetic group of 60- to 70-year-old patients. As a result, bilirubin is associated with diabetes, which was identified to affect BMD. Biochemistry biomarkers reflect the dynamic process of bone metabolism. Products of bone formation and bone absorption are released into the circulatory system, such as bone-specific ALP and osteocalcin. ALP showed negative relevance in a domestic study, and we showed the same result. Fasting blood glucose is also associated with diabetes and affects BMD. Goo et al. reported that osteoporosis results in significantly lower iron values compared with those in a normal group. We found a similar result. In addition, Goo et al. reported the results of an analysis of the prevalence of hypertension showing that 64.1% of their normal group had a normal blood pressure and 18.5% had hypertension; the values for their osteoporosis group were 45.0% and 34.3%, respectively, and those for their osteopenia group were 23.5% and 63.5%, respectively. So the ratio of hypertension seems to be increasing toward the osteoporosis group. Na studied adult women in the Seoul area and reported that blood pressure in a bone risky group was higher than that in a normal group. Lee studied the correlation between bone density and blood pressure and reported that systolic blood pressure and diastolic blood pressure are correlated. Results from these studies were consistent with our results. Taken together, the factors predictive of BMD in the present study were BMI; maximum blood pressure; minimum blood pressure; ALP, fasting blood glucose, iron, and total bilirubin levels; and neutrophil, monocyte, and eosinophil counts. As osteoporosis is a representative disease that occurs in elderly women due to ageing and menopause, more attention should be paid to prevention and treatment. We did not identify a direct cause for the decreased BMD in the subjects; thus, large-scale prospective studies are required.

ACKNOWLEDGEMENTS

Jae-Hwan Cho and Min-Tae Kim equally contributed to this work. They are co-authors of this first. This work was supported in part by the Soonchunhyang University Research Fund.

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