ORIGINAL ARTICLE

Assessment of femoral neck fractures in the elderly with respect to morphology and mineral density

H.Ç. Sorkun[1], A.H. Yay[2], E. Balcıoğlu[2], S. Özdamar[2]

[1]Denizli Health Services Vocational School, Pamukkale University, Denizli, Turkey
[2]Department of Histology and Embryology, Medical Faculty, Erciyes University, Kayseri, Turkey

Background: Femoral neck fractures are among the major orthopaedic problems seen in the elderly and the annual mortality rate is high. The calcium (Ca) and phosphorus (P) ratio can be used as an indicator of osteoporosis. The purpose of this study is to investigate the microarchitectural structure of the fractured regions of femoral head as well as bone mineral density in female and male patients.
Materials and methods: The bone tissues taken from the fractured regions of 10 male and 9 female patients were examined with a scanning electron microscope. Electron probe microanalyses were carried out to measure mineral ratios.
Results: The bone trabeculae in the fractured area were thin and the cavities between trabeculae were seen to have transformed to irregular and broad structures. There were small valleculae reflecting osteoclastic activity. The analysis showed that the Ca/P ratio at the fracture site averaged 2.20/1 in women and 2.16/1 in men. As age increased, the percentage values of Ca and P decreased and the Ca/P ratio increased.
Conclusions: Although there is no significant difference between the parameters of male and female patients, it seems that men can be affected by osteoporosis as much as women. (Folia Morphol 2016; 75, 4: 536–542)

Key words: femoral head, osteoporosis, calcium (Ca), phosphorus (P), scanning electron microscope

INTRODUCTION

Osteoporosis is defined as a systemic skeletal system disease that increases the risk of bone brittleness or causes fractures as a result of low bone mass and impaired microarchitectural structure of the bone tissue [8, 23, 29, 34, 39, 44]. Osteoporosis is the most frequently seen bone disease. It affects 1 in 3 women older than 50 and 1 in 5 men older than 50 [10]. Accidents and injuries are the fifth most frequent cause of death in older patient group after cardiovascular diseases, cerebrovascular diseases,

respiratory tract diseases and cancer [36]. Annual mortality has been reported to be between 24% and 29% in patients with fractured hips [28]. With lengthening of human lifespan, the rate of osteoporotic fractures increases.

We see that the studies made on pathogenesis, diagnosis and treatment of osteoporosis and bone brittleness focus more on women. However, epidemiological studies show that osteoporosis is an important clinical problem also in men. Although osteoporosis and resulting fractures are seen less

Address for correspondence: H.Ç. Sorkun, PhD, Associate Professor, Denizli Health Services Vocational School, Pamukkale University, 20070, Denizli, Turkey, tel: +90 5365651277, fax: +90 258 2964468, e-mail: hcsorkun@pau.edu.tr

frequently in men than in women, its prevalence is around 25% in men [8, 21].

Osteoporosis may develop as a result of pathogenetically low bone mass, excessive resorption of bones or impaired bone formation [37]. One of the methods that best reveals a high fracture risk is a bone mineral density (BMD) measurement [15]. The major components of hydroxyapatite [$Ca_5(PO_4)_3(OH)$], the bone mineral building stone, are calcium (Ca) and phosphorus (P). While Ca and P have been known as the main mineralisation support of the bone tissue, a hypothesis was brought forward stating that it also formed a potential mechanism responsible for low bone mass. It has been argued that the Ca/P ratio can be used as an indicator of osteoporosis and it will render a reliable result in the diagnosis of bone disorders [18, 38, 41]. Measuring the Ca/P ratio and finding BMD may improve our knowledge of the changes in bone material quality in patients who had a fracture or has a risk of fracture. There is not sufficient data on the changes in bone turnover in men with aging [14]. It has been stated that bone loss increases in the elderly men through aging and bone formation cannot compensate this [43] and this situation together with the change in BMD increases the risk of fractures.

The purpose of this study is to contribute to the prevention of possible bone fractures, the treatment of them and the development of appropriate protective treatment methods by determining the structure of the bone at fracture sites and the BMD values using a scanning electron microscope (SEM) in patients who had an osteoporotic femoral head fracture, which is considered as an important health problem in men, and comparing the data obtained with those of the female patients in the same age group.

MATERIALS AND METHODS

Patients

The femoral head samples used in this study were obtained after their operations from ten male patients aged between 70 and 95 and nine female patients aged between 63 and 83 who were hospitalised at the Orthopaedics and Traumatology Units of Erciyes University, Gevher Nesibe Medical School Hospital due to femoral neck fractures. Care was taken not to include any patients with chronic diseases.

SEM procedures

Since the mineral density could vary in different areas of bone tissue, only the femoral neck fracture sites were investigated. Tissue pieces sized 1 cm^3 were taken with the help of a fretsaw from the fractured parts of femoral necks that were resected and they were kept in dodecyl sulphate (1% w/v, at 37°C for 48 h), chloroform (for 48 h) and diethyl ether (for 24 h) to remove their soft tissues and fat from the bone tissue. The bone tissue samples that were left for drying for 48 h at room temperature were then plated with gold-palladium (20 nm) in a sputter coating device and examined under a LEOV-440 SEM (Erciyes University, Technology Research and Implementation Centre) at 20 kV (in secondary electron mode). Their electron probe microanalyses were carried out using an energy-dispersive X-ray spectroscopy (EDS) detector and the Ca and P values of the bone tissues were measured as percentages [33].

The patient data were analysed with the t-test and Mann-Whitney U test on the SPSS Statistics for Windows, Version 17.0 p values less than 0.05 were considered statistically significant.

RESULTS

From the patients who presented to the hospital with a femoral neck fracture, the 10 male patients had a mean age of 83.40 ± 7.07 and the 9 female patients had a mean age of 74.11 ± 6.11. The neck fracture sites of all the femoral samples examined had a cancellous bone structure. The bone trabeculae in the fracture sites anastomosed with each other and these trabeculae were rather thin. The cavities between the trabeculae were in the form of irregular and broad structures in both female and male patients. The bone tissue and trabecular structures looked similar to each other in the images taken from the fracture sites of female and male patients (Figs. 1, 2). Scanning of sections taken from the fracture sites showed small valleculae in this region. These valleculae, which were localised on the bone tissue surrounding the marrow cavities at the cancellous bone region, were found to be the resorption areas reflecting an osteoclastic activity. These valleculae in the resorption sites were adjacent to each other and had circular or oval forms. The resorption areas had no fractured regions and there were no trabeculae on this region (Fig. 3). The number of patients who had resorption areas was close to each other in female and male patients (3 in 10 male patients and 3 in 9 female patients).
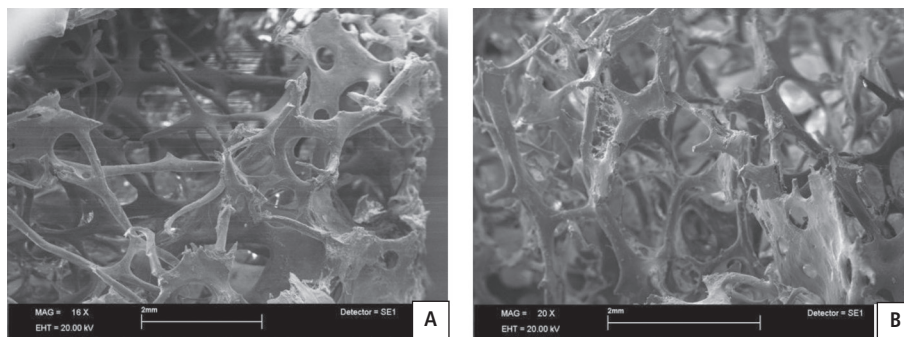
Figure 1. Broad cavities and thinned trabeculae in the cancellous bones of the fractured sites of two female patients, one aged 63 (**A**) and the other 83 (**B**).
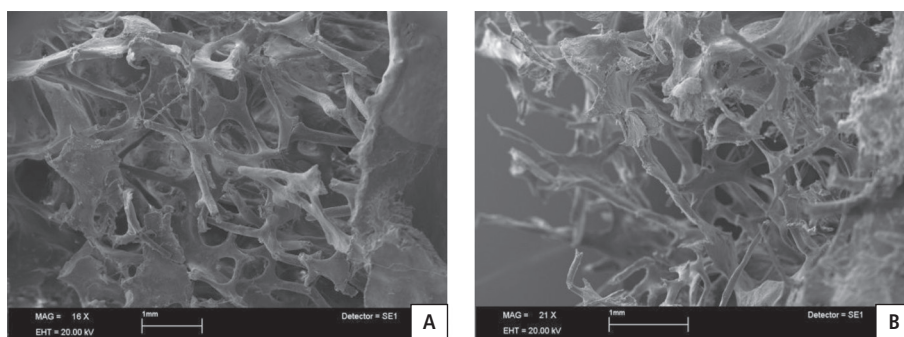

Figure 2. Thin trabecular structures and broad cavities in the femoral head fractures of two male patients, one aged 70 (**A**) and the other 89 (**B**).
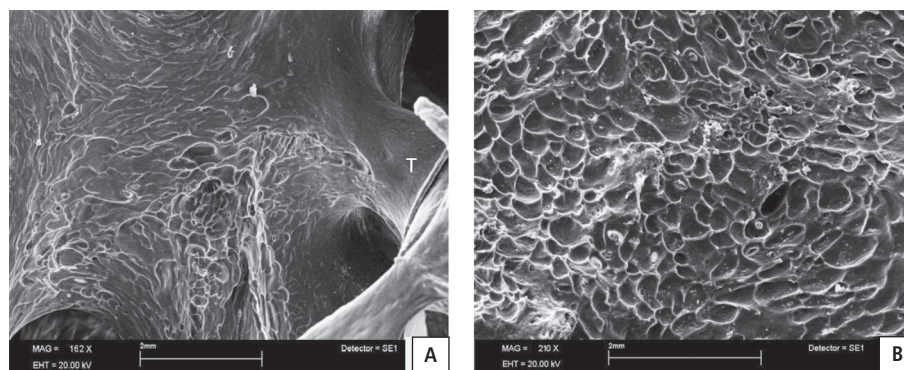

Figure 3. Valleculae reflecting osteoclastic activity on the fractured surfaces of bone tissues of male (**A**) and female (**B**) patients; T — trabecula.

The analyses showed that the mean Ca value at the fracture site was 28.81 ± 6.38% and the mean P value was 13.06 ± 4.41% in women. The overall mean Ca/P ratio was found to be 2.20/1 in women. The mean Ca value was 28.85 ± 5.77% and the mean P value was 13.35 ± 3.34% in men. The overall mean Ca/P ratio was found to be 2.16/1 in men (Table 1). When the mean Ca and P values for women and men were compared, no statisti- cal significance was found. According to the data available, the mean Ca and P values of women in the 70–79 age interval (Ca = 28.69 ± 6.57% and P = 13.43 ± 4.07%) were compared with the mean Ca and P values of men in the same age interval (Ca = 32.69 ± 1.99% and P = 16.34 ± 0.83%), but the difference was not statistically significant. When the mean Ca and P values of women in the age interval of 80–89 (Ca = 25.41 ± 5.48% and

Table 1. Age, calcium (Ca) and phosphorus (P) and Ca/P ratio pertaining to male and female patients with femoral head fractures

	Men				Women			
	Age	Ca (%)	P (%)	Ca/P	Age	Ca (%)	P (%)	Ca/P
	70	30.66	15.57	1.96/1	63	36.34	19.02	1.91/1
	76	32.78	16.22	2.02/1	70	28.51	15.08	1.96/1
	79	34.65	17.23	2.01/1	71	34.09	17.10	1.99/1
	83	37.16	17.43	2.13/1	71	18.21	7.88	2.31/1
	83	27.92	12.42	2.24/1	75	37.14	18.37	2.02/1
	85	26.14	12.80	2.04/1	75	27.70	10.61	2.61/1
	86	29.88	14.22	2.10/1	79	26.49	11.54	2.29/1
	88	17.34	7.92	2.18/1	80	21.54	7.58	2.84/1
	89	29.24	10.47	2.79/1	83	29.29	10.44	2.80/1
	95	22.79	9.31	2.44/1				
Mean	83.40	28.85	13.35	2.16/1	74.11	28.81	13.06	2.20/1

Table 2. Calcium (Ca) and phosphorus (P) values of male and female patients in their 70s and 80s

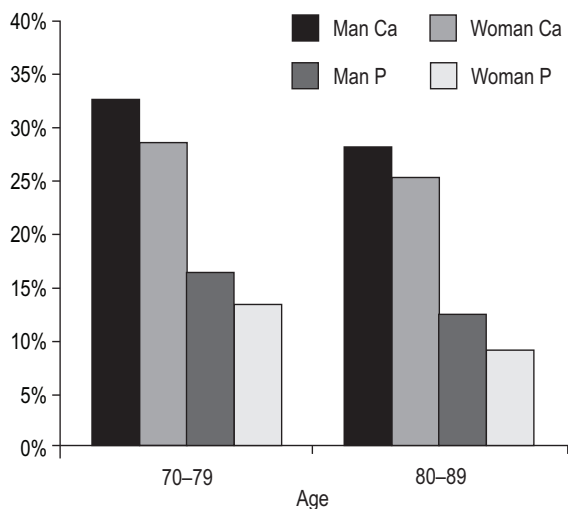| Age	Men			Women		
	Ca (%)	P (%)	Ca/P ratio	Ca (%)	P (%)	Ca/P ratio
70–79	32.69 ± 1.99	16.34 ± 0.83	2.00/1	28.69 ± 6.57	13.43 ± 4.07	2.13/1
80–89	27.94 ± 6.41	12.54 ± 3.23	2.22/1	25.41 ± 5.48	9.01 ± 2.02	2.82/1

Figure 4. Mean calcium (Ca) and phosphorus (P) values and Ca/P ratios at the femoral heads of male and female patients.
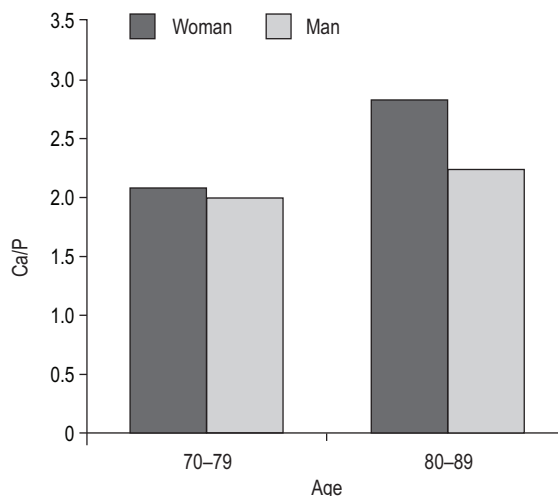
Figure 5. Calcium (Ca) and phosphorus (P) ratios in male and female patients with respect to their mean Ca and P values.

P = 9.01 ± 2.02%) were compared with those of men in the same age interval (Ca = 27.94 ± 6.41% and P = 12.54 ± 3.23%), again no statistical significance was found (Table 2). When the mean Ca and P values of patients of the same gender in their 70s and 80s were compared, the difference was not found statistically significant. The amounts of Ca and P at the

femoral head showed a decline both in women and men as age increased in general (Fig. 4). Although the amounts of Ca and P decreased, the Ca/P ratio increased in the direction of Ca as age advanced. In fact, the mean Ca/P ratio was 2.13/1 in women in the 70–79 age interval and 2.82/1 in those in the 80–89 age interval. The mean Ca/P ratio was 2.0/1 in

men in the 70–79 age interval and 2.22/1 in those in the 80–89 age interval (Fig. 5, Table 2).

DISCUSSION

Osteoporosis is an important metabolic bone disease seen frequently in the elderly [15]; it is characterised by low bone mass, excessive resorption of bones or impaired bone formation [23, 37]. Femoral head and hip fractures associated with osteoporosis are frequently seen in the elderly [34]. For this reason, a large number of morphological and quantitative studies have been carried out concerning proximal femoral fractures. The incidence of osteoporosis-related proximal femoral fractures increases in the elderly as their age advance [5]. The main reason for the occurrence of a fracture is increased bone loss. Bone loss starts around age 50 both in women and men and it increases as age advances especially after age 80 [5, 40]. In a study conducted in Turkey, it has been stated that most of the fractures in women occur after age 75 [42]. The femoral tissue samples examined in our study were taken from female patients aged 63–83 and male patients aged 70–95, the periods in which fractures are seen mostly.

A large portion of the researches made on osteoporosis and bone fractures have been performed on women [3, 4]. There is a limited number of data on the bone turnover changes associated with aging in men [14]. While only 30% of all hip fractures and 20% of all vertebra fractures occur in men [12], the rate of morbidity as well as the rate of mortality after an osteoporotic fracture is higher in men than in women [13]. In the light of these data, we considered that it would be useful if we investigate bone turnover in aged men and the osteoporotic effect on it and evaluate these clinically. Therefore, we examined the morphology and mineral density of the femoral tissue samples taken from the fractured regions of male patients who presented with a fracture using SEM and EDS and compared them with the samples from female patients in similar age groups.

Data on bone building and resorption in elderly men are conflicting [43]. In order to assess the risk of brittleness in men, bone quality and factors affecting bone strength should be identified. Various methods such as bone microstructure, reshaping and strength are used to assess bone diseases. Histomorphometric studies on osteoporotic bones have shown decreased bone volume, low trabecula count, reduced trabecular volume and increased distance between trabeculae [30]. The effect of impaired balance in osteoblastic and osteoclastic activities is considerable [7] on the occurrence of low bone density or excessive bone resorption [34]. While the osteoblastic activity is normal in 37% of women in their post-menopause periods, there is an increase in their osteoclastic activity [26]. We observed the areas that show the presence of osteoclastic activity on the surfaces of fractured regions in our study. The structural characteristic of osteoclastic surfaces is that such regions may form microcracks. Osteoclastic lacunae are in the form of interlinked structures and there were no trabeculae in these areas. This observation is similar to the results of Li et al. [24] and Lozo et al. [26]. Hordon et al. [19] reported in their study on vertebra fractures that there were less trabeculae and they were in a separated form, but trabeculae were thicker although not significantly. It was reported in the study of Leszczyński et al. [22] that the trabecular separation increased tremendously with age while the percent bone volume was considerably reduced on lumbar vertebra, but trabecular thickness was not altered by the aging process. Chai et al. [7] have stated that broad and irregular cavities in the femoral neck region reduce bone strength and facilitate fractures. We also observed in the images obtained from fractured areas in our study that the trabecula structures were thinner and the bone marrow cavities were widened. This characteristic can be part of the bone's brittleness. There was not any histological difference between the fractured regions of female and male patients in terms of bone density, trabecula size, or cavity width.

Measuring bone volume/trabecula volume and Ca amount alone is not sufficient in determining bone quality; the P value should also be measured. Alongside Ca and P values, their ratio with respect to each other, Ca/P, is also important in assessing fractures. A change in the Ca/P ratio in favour of Ca will affect bone quality and this may increase the risk of brittleness by increasing the crispiness of the bone. Not a single type mineralisation structure is seen in the elderly due to different mineralisation levels and variations in remodelling [25, 31]. Increased homogeneity in the bone tissue and the bone becoming hardened and crispy may increase the risk of bone fracture. However, Busse et al. [6] compared fractured and healthy bones and found that the central regions with high mineral content being surrounded by surfaces with low mineral content increased the stability and firmness of a bone. They did not observe any difference between firm and brittle bones with respect to mean Ca percentages. Fratzl-Zelman

et al. [16] and Roschger et al. [32] have shown that there is a high bone turnover with changing BMD values in osteoporotic men and have stated that the increased mineralisation rate in these patients may be a response to protect the bone's own strength. However, Fratzl-Zelman et al. [17] have also reported in another study of theirs that brittleness in men is associated with low mineralisation. Basle et al. [1] have compared the Ca/P ratio in their control group and osteoporotic group of men in the 31–87 age interval and have shown that there is no difference in the Ca and P concentrations in the cortical and trabecular bones. In the present study, we evaluated patient groups by their genders and age groups without using any control group. We found that the Ca and P values of those in the 80–89 age group were lower than those in the 70–79 age group both in men and women. We also observed that when the same age groups were compared, the Ca/P ratio increased in favour of Ca at advanced ages. While these results are similar to those of Kim et al. [20] and Maharlouei et al. [27], they contradict the results of Basle et al. [1]. The reason for this inconsistency may be because Basle et al. [1] studied a broad age interval including ages from 31 to 87. The age interval in our study was from 63 to 95.

The balance between the firmness and elasticity of a bone is determined by the change in its mineral content [18, 35, 38, 41]. As the mineral content increases, the firmness of the material increases and its elasticity decreases [9]. Studies have reported that a hypermineralised bone tissue increases the risk of a fracture in femoral neck at advanced ages [2, 11, 39]. Li et al. [24] have shown that the Ca/P ratio is 1.67 in healthy individuals. Our results indicate that the average Ca/P ratios are 2.20/1 in women and 2.16/1 in men at the fracture site. These ratios show that the bone tissues of examined patients were more mineralised than normal. There are conflicts between the previous studies carried out on the Ca and P concentrations and Ca/P ratio in osteoporosis. Our data demonstrate that the change in Ca and P values and trabecula structure are related to bone quality and bone strength. The results give evidence that the bone Ca/P ratio can be a valuable parameter to be used during bone treatment and diagnosis works. Since the time it was implemented successfully in animals [43], it is exciting that in vivo measurements of the bone Ca/P ratio [14] will be possible for humans in the future.

CONCLUSIONS

In conclusion, our results show that bone quality decreases with advancing age. Reduced bone volume and trabecula thickness as well as osteoclastic activity and resulting microcracks increase the risk of a fracture. Diminishing Ca and P values and impaired mineral balance in favour of Ca make the bone crispy and increase brittleness. There was no significant difference between the parameters of male and female patients we had examined; men can be affected by osteoporosis as much as women.

Since it progresses clinically without a sign until a fracture occurs, men who have risk factors for osteoporosis and who actually have osteoporosis should be identified. Once osteoporosis is diagnosed, these persons can be treated and in this way morbidity and mortality associated with osteoporotic fractures can be minimised. There is a need for further studies that would make us understand the role of mineralisation breakdown in bone brittleness and show the variability of mineralisation breakdown among osteoporotic individuals. We think that such studies should be carried out with people of the same gender within limited age groups and on a comparative basis.

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