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Bone Mineral Density of the Spine and Femur in Early Postmenopausal Turkish Women with Endemic Skeletal Fluorosis

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Abstract. The aim of this prospective, comparative study was to investigate the bone mineral density (BMD) changes in a group of early postmenopausal Turkish women with endemic skeletal fluorosis and to study effects of endemic fluorosis on BMD. Bone mineral density of L_2–L_4 vertebra, femur neck, femur trochanter, and Ward's triangle were measured in 45 female patients with endemic skeletal fluorosis and 41 age-matched controls by dual X-ray absorbtiometry (DXA). The BMD of L_2–L_4 vertebra and Ward's triangle were higher in the endemic fluorosis group than in the control group ($P < 0.001$). Patients with endemic fluorosis had higher femur neck and femur trochanter BMDs than did controls ($P < 0.01$ and $P < 0.05$, respectively). There was a positive correlation between serum fluoride content and BMD at the spine ($r = 0.345$, $P = 0.001$), femoral neck ($r = 0.274$, $P = 0.011$), Ward's triangle ($r = 0.295$, $P = 0.006$), and trochanter ($r = 0.217$, $P = 0.045$). In conclusion, higher bone mineral density levels were seen in early postmenopausal women with endemic skeletal fluorosis. BMD measurement is a tool in the diagnosis and management of this preventable crippling disease.

Key words: Fluoride — Endemic fluorosis — Bone mineral density

Endemic skeletal fluorosis is a chronic metabolic bone disease caused by ingestion of large amounts of fluoride either through water or, rarely, from foods of endemic areas [1]. Although the prevalence of this disease has decreased considerably, it is still endemic in many places around the world such as India, China, and Africa [2–4]. This disease is a public health problem in Isparta, a country situated in the south of Turkey and located in an endemic fluorosis area. An increased number of patients with dental fluorosis have been reported to have been admitted to the university hospital complaining of knee pain [5].

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Approximately 98% of the fluoride in the body is associated with calcified tissues. Fluoride is incorporated into bones where, as a result of similarities in size and charge, it replaces the hydroxyl ion in the crystal lattice of apatite [6]. Fluoroapatite is less soluble, more compact, and slower to undergo remodeling in bone [7]. Excessive intake of fluoride may lead to pathological differences in teeth (mottled teeth) and bones (skeletal fluorosis) [8]. Fluoride is a cumulative poison that increases metabolic turnover of the bone in favor of bone formation [1, 9]. It stimulates bone cell proliferation by directly inhibiting osteoblastic acid phosphates activity [10], and by prolonging or enhancing the mitogenic signals of growth factors [11]. Histopathological studies of bone in fluorosis have shown osteoid tissue deposited irregularly on the trabeculae and cortex [12]. Endemic skeletal fluorosis is characterized by wide skeletal manifestations such as osteopenia, osteoporosis, and osteosclerosis, resulting in crippling deformities and spinal cord compression and hypertrophy of the joints and bones similar to osteoarthritis [13–18].

To our knowledge, no previous data have been reported on bone mineral density (BMD) changes in Turkish patients with endemic skeletal fluorosis. In the present study, we aimed to compare BMD values between the early postmenopausal women exposed to an elevated fluoride level in drinking water and women with normal fluoride intake.

Materials and Method

Subjects

Forty-five early postmenopausal women with clinically proven endemic skeletal fluorosis were recruited and measured for spinal and femoral BMDs. The clinical diagnosis of endemic fluorosis was modified from the criteria of Wang et al [13]: (1) living in the endemic fluorosis region since birth, (2) having mottled tooth enamel, indicating dental fluorosis, (3) consuming water with fluoride levels above 1.2 ppm (normal 1

Table 1. Characteristics of study subjects

	Endemic fluorosis n = 45	Controls n = 41	*P*
Mean age (years)	49.53 ± 4.22	51.43 ± 4.39	0.31
YSM (years)	3.2 ± 2.2	3.5 ± 2.1	0.29
BMI (kg/m^2)	26.84 ± 4.06	26.81 ± 3.73	0.77
Serum fluoride (mg/l)	0.11 ± 0.32	0.03 ± 0.01	< 0.001
Urine fluoride (mg/l)	4.15 ± 1.81	0.38 ± 0.20	< 0.001
Serum total calcium (mg/dL)	9.6 ± 1.2	9.5 ± 1.1	0.67
Inorganic phosphorus (mg/dL)	3.4 ± 0.9	3.3 ± 0.8	0.59
Alkaline phosphatase (U/L)	73.9 ± 34.2	70.41 ± 30.4	0.61
Total proteins (g/dL)	6.82 ± 0.71	6.91 ± 0.83	0.59
Albumin (g/dL)	4.0 ± 0.5	4.1 ± 0.3	0.40
Transferrin (mg/dL)	291.7 ± 41.3	294.2 ± 37.1	0.77
Prealbumin (mg/dL)	19.8 ± 5.0	21.4 ± 7.2	0.24

Values are expressed as mean ± SD
YSM: years since menopause; BMI: body mass index
Normal ranges: serum total calcium (8.2–10.9 mg/dL), inorganic phosphorus (2.7–4.5 mg/dL), aIkaline phosphatase (42–141 U/L), total proteins (6.0–8.0 g/dL), albumin (3.5–5.0 g/dL), transferrin (200–400 mg/dL), prealbumin (12–42 mg/dL)

ppm), and (4) a urine fluoride level greater than 1.5 mg/l (normal < 1.5 tng/l).

BMD measurements were taken at the lumbar spine, femoral neck, Ward's triangle, and trochanter in 41 early postmenopausal women living in a nonendemic region who were randomly selected as controls. According to Isparta Health Organization data the mean fluoride level in drinking water was 2.74 ± 0.64 ppm in the endemic fluorosis region and 0.53 ± 0.06 ppm in the nonendemic region.

All the study subjects were urbanites and housewives. None had ever smoked or had had diseases such as renal failure, hepatic disease, malabsorption, or metabolic bone or inflammatory disease. Their body weight and height were recorded. Body mass index (BMI) was calculated for each patient as weight (kg)/height $(m)^2$. The ethics committee of Süleyman Demirel University Medical Faculty approved the study protocol. Informed consent was obtained from the patients and controls.

Biochemical Measurements

Serum samples were analyzed for calcium, inorganic phosphorus, total alkaline phosphates, total proteins, albumin, prealbumin, transferrin, creatinine, and blood urea nitrogen by standard biochemical methods. Serum and urine samples were analyzed for fluoride (F) by using an ion-specific electrode (Orion F 94-09).

Bone Mineral Density Assessment

Bone mineral density (BMD) (g/cm^2) was measured at the AP lumbar spine (L_2–L_4), femur neck, Ward's triangle, and femur trochanter by DXA (Norland XR-46 bone dansitometer, with dynamic filtration, Norland Corp, Fort Atkinson, USA). The Norland XR-46 was calibrated daily, 30 min after turning the aparatus on. Quality control was performed using calibration standard and QC phantom.

Statistical Analysis

Data were analyzed using the statistical package SPSS for Windows (Ref. 9.05, SPSS Inc., Chicago, IL.). Results were given as mean ± SD. Statistical significance was set at 0.05. Comparison of the groups was performed using Student's-*t* Test and Mann–Whitney Test where appropriate. Correlation analysis was performed to assess the relation between some BMD data.

Results

The characteristics of the study subjects are shown in Table 1. There were no differences between the groups in age, duration after menopause, and BMI. Serum total calcium, inorganic phosphorus, alkaline phosphates, total protein, albumin, prealbumin, transferrin, creatinine, and blood urea nitrogen concentrations were found to be normal in all subjects. Serum and urine fluoride levels in the women with endemic fluorosis were significantly higher than in controls ($P < 0.001$).

The mean BMD of vertebra L_2–L_4, femur neck, trochanter, and Ward's triangle in the patients with endemic fluorosis were significantly higher than that of controls ($P < 0.001$, $P < 0.01$, $P < 0.01$, $P < 0.05$, and $P < 0.001$) (Table 2).

In patients with skeletal fluorosis, serum fluoride content was correlated positively with BMD at the spine ($r = 0.345$, $P = 0.001$) (Fig. 1), femoral neck ($r = 0.274$, $P = 0.011$), Ward's triangle ($r = 0.295$, $P = 0.006$), and trochanter ($r = 0.217$, $P < 0.045$). There was a positive correlation between urinary fluoride content and BMD at the spine ($r = 0.481$, $P = 0.001$) (Fig. 2), femoral neck ($r = 0.395$, $P = 0.001$), Ward's triangle ($r = 0.398$, $P = 0.001$), and trochanter ($r = 0.327$, $P = 0.002$).

Discussion

Fluoride has the potential to increase skeletal mass to a greater extent than any other pharmacological agent. Exposure of calcified tissues to environmental fluoride offers paradoxes. The anticaries effects are well documented as are the deleterious skeletal effects of endemic fluorosis. Although endemic fluorosis occurs in many countries, epidemiological studies have been limited because of funding problems, a lack of awareness of the

Table 2. BME[a] of lumbar spine and femur in women with endemic fluorosis and controls

	Endemic fluorosis n = 45	Controls n = 41	*P*
Vertebra L_2–L_4	1.0761 ± 0.179	0.8579 ± 0.1270	< 0.001
Femur neck	0.8603 ± 0.1166	0.7753 ± 0.1180	< 0.01
Femur trochanter	0.6821 ± 0.107	0.5966 ± 0.1404	< 0.05
Ward's triangle	0.6527 ± 0.1355	0.5567 ± 0.0124	< 0.001

[a] Mean ± SD (g/cm^2)

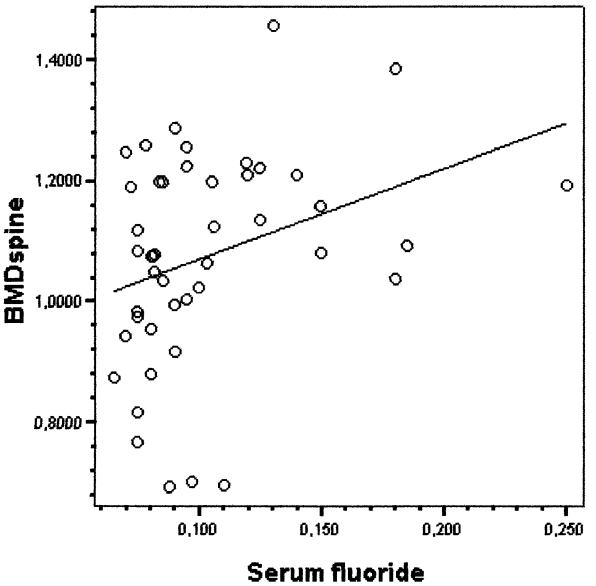

Fig. 1. Serum fluoride content was correlated positively with BMD at the spine (r = 0.345, *P* = 0.001).

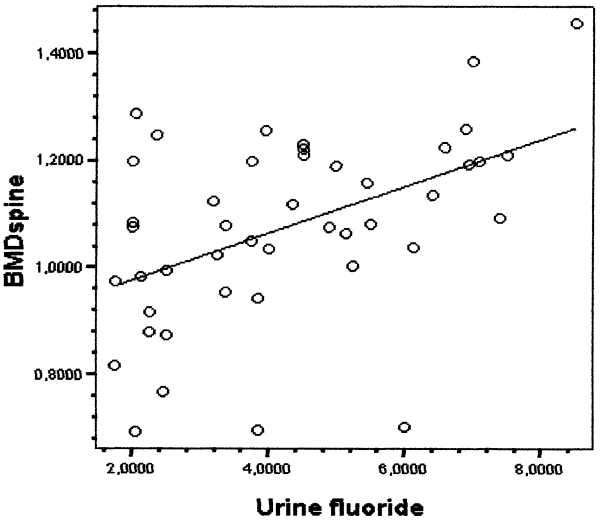

Fig. 2. Urine fluoride content correlated positively with BMD at the spine (r = 0.481, *P* = 0.002).

disease as a public health problem, and the absence of inexpensive defluoridation methods. Water is the major source of fluoride ions in Isparta where skeletal and dental fluorosis are endemic. Although overfluoridated drinking water in Isparta was diluted with some non-fluoridated water sources with the object of supplying safe and sufficient water to the entire population, the fluoride levels of some drinking water are still high (2.7 ppm). Most of the studies have reported 1 ppm of sodium fluoride to be a safe level in drinking water [19]. A wide range of skeletal changes such as osteoporosis, osteopenia, and osteosclerosis have been described in endemic skeletal fluorosis [13–15]. The causes for this differences may be due to several factors such as dose, duration of fluoride exposure, age, dietary habits or a combination of factors [1, 2, 20, 21]. The total quantity of fluoride intake is reported to be the most important factor contributing to the skeletal abnormality of this disease. Czarnowski et al. [22] reported that subjects exposed to an elevated fluoride level (about 3 ppm) in drinking water have higher bone mineral density compared with controls. Lan et al. [23] showed that the BMD of the premenopausal women from an area with a fluoride dose > 1 mg/1 in the drinking water in Taiwan was significantly higher than that from the reference group (fluoride < 0.6 mg/l). Phipps et al. [24] compared women with continuous exposure to fluoridated water for the past 20 years in the United States with women who had no exposure, and demonstrated higher BMDs at the femoral neck and vertebra, and lower BMDs at the distal radius. Sogaard et al. [21] suggested that when much higher levels of fluoride (100–150 ppm) were taken, BMD became high but bone quality declined.

However, the concentration of fluoride alone was not found to be responsible for the skeletal fluorosis. It is accepted that poor nutrition and low Ca intake enhance the deleterious effect of fluoride [1, 2]. Backscattered electron imaging studies by Grynpas [7] showed focal and linear mineralization defects within the bone tissue from fluoride-treated subjects. Therefore, inadequate Ca intake may result in low BMD in some skeletal fluorosis patients. In parts of India, where fluorosis is endemic, Mithal et al. [14] showed that subjects with osteopenic changes had a significantly lower dietary intake of Ca than those groups having normal radiological findings. In our study, the nutritional parameters of the study and control subjects were within the normal range, judging from serum levels of total protein, albumin, prealbumin, and transferring.

Based on the biochemical markers of nutrition, all the patients had a good nutritional status. Poor nutrition,

which can effect BMD, was not met in our study and the BMD in women with endemic skeletal fluorosis was found to be higher than that of controls. In patients with skeletal fluorosis, positive correlations were found between urinary fluoride content and BMD and serum fluoride content and BMD.

Fluoride is incorporated more readily in trabecular bone than in cortical bone [25]. Kroger et al. [26] reported that 969 women who had used fluoridated drinking water (1–1.2 ppm) for over 10 years had significantly higher BMD of the spine than the nonfluoride group, but femoral neck BMDs did not differ between the groups. However, when the BMD values were adjusted for confounding factors such as age, weight, menopausal status, and estrogen use, there was also significant difference between the groups for the femoral neck BMDs. In one study of 202 women with osteoporosis who were treated with sodium fluoride, spine BMD increased but forearm BMD decreased, indicating redistribution of bone mineral from cortical to trabecular bone [27]. In agreement with these literature findings, we found the differences of spinal BMD and Ward's area between the groups more significant than the differences in femur neck and femur trochanter. Our results show that BMD values in postmenopausal endemic fluorosis patients were significantly higher than postmenopausal control subjects due to the continuous fluoride exposure, but this difference was more prominent in spinal BMD and Ward's area, indicating redistribution of bone mineral from cortical to trabecular bone.

Fluoride is known to stimulate bone formation and increase bone mass. Therefore it has been used throughout the world for the treatment of osteoporosis for 4 decades. However, some recent clinical trials failed to prove its antifracture effectiveness. In fact, the increase in bone mass during fluoride treatment does not translate into improved bone strength. In one experimental study investigating the effects of fluoride intake on bone, it was shown that fluoride treatment increased bone mass, but decreased bone strength [28]. In human trials, sodium fluoride has not been shown to decrease the number of new vertebral fractures [29–32]. Moreover, the risk of nonvertebral fractures may have been somewhat higher in the trials using a relatively high daily dosage of sodium fluoride, 75 mg [27].

In conclusion, we suggest that the early postmenopausal women with endemic skeletal fluorosis have high BMD levels. However, further studies are needed to show the effect of increased BMD levels on bone fracture risk in this group of patients.

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