BONE RESORPTION MARKER AND ULTRASOUND MEASUREMENTS IN ADULTS RESIDING IN AN ENDEMIC FLUOROSIS AREA OF TURKEY

Oya Topuz,[a] Nuray Akkaya,[a] Füsun Ardıç[a] Ayşe Sarsan,[a] Duygu Çubukçu,[a] Ali Gökgöz[b]

Denizli, Turkey

SUMMARY: The purpose of this prospective study was to investigate the quantity and quality of bone by quantitative ultrasound (QUS) measurements and to assess bone resorption by urinary excretion measurement of C-terminal telopeptide of type I collagen (CTX) in an adult Turkish population living in an endemic fluorosis area and consuming drinking water with a high fluoride (F) concentration (mean 3.57 ppm F). Excretion of urinary CTX, heel broadband ultrasound attenuation (HBUA; dB/MHz), and speed of sound (SOS; m/s) were examined in 122 Turkish adults (37 pre-menopausal, 40 post-menopausal women, and 45 men) living in the endemic fluorosis area. For comparison, the same measurements were made on 117 controls (48 pre-menopausal women, 34 post-menopausal women, and 35 men) living in a nonendemic low F water area (mean 0.4 ppm F). In the F endemic area urinary excretion of CTX was higher in all subjects, whereas calcaneal BUA was lower in post-menopausal women. In the F endemic area SOS was significantly greater among pre-menopausal women but was not significantly different in the other two groups. Although non-trauma bone fracture rates were not significantly different among any of the groups, some of the bone marker differences indicate that exposure to prolonged high concentration of F may increase the risk of bone fracture, especially in post-menopausal women.

Keywords: Bone resorption; Endemic fluorosis; Heel broadband ultrasound attenuation (HBUA); Quantitative ultrasound (QUS); Speed of sound in bone; Turkish adults; Urinary C-terminal telopeptide (CTX).

INTRODUCTION

Endemic skeletal fluorosis is a chronic metabolic bone disease caused by ingestion of elevated amounts of fluoride (F) either through water or, less often, from foods in affected areas.[1] SF is still endemic, especially in India, China, and Africa.[2-4] In some regions of Western Anatolia, Turkey, groundwater is the major source of drinking water; in Ortakçı, a village near the city of Denizli, the F level of drinking water is unusually high.

Reports in the literature on the risk of bone fracture associated with long-term exposure to F in drinking water are contradictory. Some indicate an increased risk,[5-9] others no effect,[10-13] and still others a decreased risk.[14,15] However, the risk of fracture is strongly related to bone mineral density (BMD), which is usually measured by dual-energy X-ray absorptiometry (DXA).[16-18] Currently, there is increasing interest in the use of quantitative ultrasound (QUS) measurements of the heel (calcaneus) bone for prediction of fracture risk. It has been suggested that the parameters of QUS, broadband ultrasound attenuation (BUA), and speed of sound (SOS) depend not only on bone density but also on bone structure and elasticity.[19,20] Fracture risk is considered to be higher in individuals with high

[a]For correspondence: Dr Oya Topuz, Çamkent Sitesi, No. 49 Çamlık, Denizli, Turkey; E-mail otopuz@pamukkale.edu.tr; [a]Pamukkale University, Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Denizli, Turkey; [b]Pamukkale University, Faculty of Engineering, Department of Geological Engineering, Denizli, Turkey.

bone turnover. C-terminal telopeptide of type I collagen (CTX) is a bone resorption marker excreted by urine. Post-menopausal women with higher levels of urinary CTX excretion are reported to have twice the risk of fracture compared to women with normal urinary CTX excretion, even after adjustment for bone density.[21]

To our knowledge, QUS measurements of the heel bone and urinary excretion of CTX in adults living in endemic fluorosis area have not been reported previously. The purpose of this prospective study was to investigate bone quantity and quality with QUS measurements of the heel bone, and to assess bone resorption with urinary excretion measurement of CTX in an adult Turkish population consuming drinking water with high levels of F and comparing the results with the individuals consuming normal low F water. aa

MATERIALS AND METHODS

The study population consisted of 122 subjects (37 pre-menopausal women, 40 post-menopausal women, and 45 men) living in endemic fluorosis area in the village of Ortakçı in Western Anatolia, Turkey, matched for age, sex, and menopausal status with 117 controls (48 pre-menopausal women, 34 post-menopausal women, and 35 men) living in a non-endemic area of the city of Denizli. According to the Mother & Child Health and Family Planning Department of the Turkish Ministry of Health,[22] the mean F level in drinking water in the non-endemic regions of Denizli is 0.40 ppm. For Ortakçı village the mean F level in the drinking water was determined as 3.57 ppm using a HACH DR/4000 visible spectrophotometer. The clinical diagnosis of endemic fluorosis was made according to the criteria of Wang et al.[23] modified for subjects: (1) living in the endemic fluorosis region since birth, (2) having mottled tooth enamel, indicating dental fluorosis, and (3) consuming water with fluoride levels above 1.2 ppm. The residency of each individual was confirmed by the village official who was familiar with the resident.

Data collected from subjects included demographic information, detailed medical history, physical activity, smoking, and consumption of alcohol, tea, and dairy products. Records of bone fractures in the medical histories of the patients and the numbers of fractures in each patient were also recorded. Persons with fractures clearly due to major trauma, such as motor vehicle accidents or local pathology (e.g., cancer), were excluded from the study. In this study, subjects with dental fluorosis were not assessed with respect to skeletal fluorosis.

After exclusion of patients with metabolic bone-related diseases (e.g., primary hyperparathyroidism, kidney dysfunction requiring chronic hemodialysis., etc.) or any treatment (bisphosphonate, F, calcium, and vitamin D_3) known to influence calcium metabolism, measurements of heel broadband ultrasound attenuation (BUA; dB/MHz) and speed of sound (SOS; m/s) were made along with collection of first morning urine samples. The measurements of BUA and SOS in the left calcaneus were performed with an ultrasonographic densitometer (Hologic Sahara Clinical Bone Sonometer, Waltham, MA, USA). Quality was assured by daily calibration using the phantom supplied by the manufacturer.

Urinary CTX was measured by Osteosal (Provalis Diagnostics, UK), designed for use in conjunction with InstaQuant, a purpose-built hand-held photometer that measures Cross-Laps antigen in urine. CrossLaps is a bone resorption marker and is the eight amino acid sequence found on the C-telopeptide of type 1 collagen. The test results with Osteosal are expressed as a T score that defined as the number of standard deviations of the patient's result from the mean of the normal pre-menopausal population. Body mass index (BMI) was calculated for each subject as weight (kg) divided by squared meters of height. Dental fluorosis of the subjects living in the endemic fluorosis area was determined according to Dean's Index.[24]

Groups were compared by using Student's t test in parametric values and the Mann Whitney-U test in non-parametric values. Results are given as mean ±SD. For the statistical evaluation of fracture history, chi-square tests were performed, and results are given as percentages. Any correlations between severity of dental fluorosis and bone findings were evaluated by using Spearman's Correlation Test. Statistical significance was set at the 0.05 level.

RESULTS

Among all the groups there were no differences in the weekly consumption of tea, coffee, alcohol, and dairy products. In the pre-menopausal and post-menopausal endemic fluorosis area groups, smoking was significantly less than in the corresponding control groups (p=0.004, p=0.04, respectively). In the men in the endemic fluorosis area, smoking was significantly higher compared to the control group (53.3% and 25.7%, respectively; p=0.02). In the pre-menopausal and post-menopausal endemic fluorosis area groups, women were more physically active compared to their corresponding control groups (p=0.0001). In all the endemic fluorosis area groups, the subjects were less educated compared to their corresponding control groups (pre-menopausal and men groups, p=0.0001; post-menopausal group, p=0.02). There was, however, a positive correlation between the severity of dental fluorosis and urinary CTX (r = 0.228, p=0.001). In all subjects, there were no significant differences between the groups in age, BMI, and history of fracture. However, the mean urinary CTX value in all subjects in the endemic fluorosis area was significantly higher compared to the controls. The characteristics and results of calcaneal ultrasound and urinary CTX measurements of pre-menopausal women, post-menopausal women, and men in the endemic fluorosis area, along with those of their controls, are given in Tables 1, 2, and 3, respectively.

Table 1. Data for pre-menopausal women residing in endemic fluorosis area plus controls

	Endemic F area N= 37	Controls N=48	P
Age (years) (mean±SD)	40.21 ± 5.23	41.09 ± 5.80	0.46
BMI (kg/m^2) (a)	26.40 ± 3.86	26.98 ± 4.98	0.52
SOS (m/s) (mean±SD)	1568.16 ± 22.76	1553.26 ± 31.03	0.01*
BUA (dB/MHz) (mean±SD)	73.64 ± 13.85	73.37 ± 19.03	0.93
CTX (Tscore) (mean±SD)	1.97 ± 1.38	0.04 ± 2.42	0.00*
Subjects with history of fracture (%)	3 (8.1)	5 (10.4)	0.67
Subjects with history of one fracture (%	3 (8.1)	4 (8.3)	
Subjects with history of two fractures (%)	0 (0)	1 (2.1)	

*Statistically significant as indicated.

Table 2. Data for post-menopausal women residing in endemic fluorosis area plus controls

	Endemic F area N= 40	Controls N=34	P
Age (years) (mean±SD)	56.37 ± 6.76	54.55 ± 7.07	0.26
BMI (kg/m^2) (mean±SD)	25.62 ± 3.07	26.42 ± 2.93	0.26
Duration of menopause (years) (mean±SD)	11.27 ± 7.51	8.52 ± 7.46	0.12
SOS (m/s) (mean±SD)	1539.29 ± 27.90	1545.12 ± 27.99	0.37
BUA(dB/MHz) (mean±SD)	61.21 ± 16.57	69.55 ± 17.61	0.04*
CTX (Tscore) (mean±SD)	2.27 ± 1.22	0.13 ± 1.61	0.00*
Subjects with history of fracture (%)	7 (17.5)	7 (20.6)	0.29
Subjects with history of one fracture (%)	5 (12.5)	7 (20.6)	
Subjects with history of two fractures (%)	2 (5)	0 (0)	

*Statistically significant as indicated.

Table 3. Data for men residing in endemic fluorosis area plus controls

	Endemic F area N= 45	Controls N=35	P
Age (years) (mean±SD)	52.35 ± 13.85	50.60 ± 10.74	0.52
BMI (kg/m^2) (mean±SD)	25.41 ± 4.04	25.89 ± 3.38	0.57
SOS (m/s) (mean±SD)	1554.35 ± 34.29	1545.61 ± 29.87	0.24
BUA(dB/MHz) (mean±SD)	75.91 ± 22.73	72.61 ± 29.87	0.48
CTX (Tscore) (mean±SD)	2.33 ± 1.63	1.43 ± 1.80	0.02*
Subjects with history of fracture (%))	9 (20)	9 (25.7)	0.48
Subjects with history of one fracture (%	8 (17.8)	9 (25.7)	
Subjects with history of two fractures (%)	1 (2.2)	0 (0)	

*Statistically significant as indicated.

DISCUSSION

Drinking water is a significant source of daily F intake, especially in localities with relatively high F concentrations in the domestic and municipal groundwater. In the World Health Organization's *Guidelines for Drinking-Water Quality*, a maximum of 1.5 mg F/L is recommended, accompanied by a statement that low concentrations of F in drinking water offer protection against dental caries, especially in children.[25] In some countries, water fluoridation has been adopted as a method to reduce the prevalence of dental caries, but its effectiveness to reduce tooth decay has come under increasing doubt. Although a caries deterrent effect of topical F is widely accepted, ingested F appears to have little prophylactic benefit against tooth decay, and elevated intake is well documented to have adverse effects on tooth enamel as well as skeletal tissues.[26,27]

Studies examining the radiographic features of patients with skeletal fluorosis reveal signs of osteosclerosis, osteomalacia, and osteoporosis.[9,23,28] Some reports indicate that F stimulates bone formation and increases in bone mass,[29-32] but Krook and Minor[33] suggest that the increase in serum alkaline phosphatase level after F therapy may reflect the toxic effects of F on both osteoblast and resorbing osteocytes, arguing that F-induced cell injury initiates a repair response. They propose that when the repair process fails, osteoblast and resorbing osteocytes undergo either apoptosis or necrosis. In their view the decrease in bone turnover may be enhanced by a secondary F-induced injury of osteoclasts that are formed to

degrade the necrotic bone. Thus F treatment fails to improve bone strength and bone quality declines.[34-36]

Because broadband ultrasound attenuation (BUA) and speed of sound (SOS) measurements on bone depend not only on bone density but also on bone structure and elasticity,[20,37] our study was focused quantitative ultrasound (QUS) parameters of BUA and SOS determinations in subjects living in a endemic fluorosis region. The SOS value represents the time it takes the sound wave to pass through bone alone. The greater the connectivity or complexity of a structure, the greater will be the velocity of the sound wave through the structure. BUA is thought to depend on the bone structure and is a function of the spatial distribution and size of the individual trabeculae. The more complex a structure, the more the sound wave passing through it will be blocked or attenuated.[38]

Our study demonstrated that consumption of high F drinking water produces an increase in cancellous ultrasound velocity in pre-menopausal women, whereas in post-menopausal women a lower BUA was found indicating a decrease in bone mass. This result can be explained by the fact that, in addition to fluorosis, menopause and age may synergistically affect bone quality. In this connection it should be noted that Turner et al.[35] found that the effects of aging and incorporation of F in bone act synergistically in rats to decrease bone strength. Similarly, Yıldız et al.[39] found that post-menopausal BMD values were significantly lower in both endemic fluorosis and control women than in pre-menopausal women, thus suggesting that menopause is the major determinant of BMD in the spine and femur. In addition, some studies indicate that high bone resorption markers reflect not only future loss in BMD (bone "quantity") but also possible defects in bone architecture (bone "quality"), and may therefore help predict risk of bone fracture.[21,40]

In our study we found that urinary CTX excretion in all the subjects residing in the endemic fluorosis area was significantly higher than in the controls. Likewise, Ando et al.[41] reported that high concentrations of F in food directly stimulate bone resorption and cause excretion of higher levels of urinary deoxypyridinoline. In an animal study, Turner et al.[36] showed that serum bone-specific alkaline phosphatase and tartrate-resistant acid phosphatase levels were significantly increased by F treatment, indicating an enhanced bone turnover.[36] Moreover, Garnero et al.[42] found that addition of a bone resorption parameter to the bone mass/quality (femoral neck BMD or heel BUA) parameters might be useful to identify elderly women at a higher risk of hip fracture.

The association of increased CTX and decreased BUA in our study led us to think that the risk of bone fracture might be greater in post-menopausal women living in an endemic fluorosis area. In this connection, Li et al.[9] in an epidemiological study found that hip fracture prevalence was stable up to 1.06 ppm F in drinking water and then increased, although it did not attain statistical significance until the F concentration reached 4.32–7.97 ppm.[9] A positive correlation between dental fluorosis and bone fractures was observed by Herrera et al.[43] as well as a paradoxical relationship between the occurrence of fractures and the F concentration in water. In our study we did not find any statistically significant difference in the fracture history between the endemic fluorosis area

and control groups. Although pre-menopausal and post-menopausal women in the endemic fluorosis area groups were more physically active than in their corresponding control groups, this difference, which may have had a beneficial effect on their bone mass, bone density, and muscle strength, did not appear to provide any protection for them against risk of bone fracture.

In conclusion, we have demonstrated that urinary CTX excretion, a bone resorption marker, was increased in pre-menopausal and post-menopausal women and men residing in an endemic fluorosis area, whereas calcaneal BUA, an assessment of bone quantity and quality, was lower only in post-menopausal women in the endemic fluorosis area. Because of the reported predictive value of urinary CTX and calcaneal BUA for risk of bone fracture, we view long-term F intake from drinking water as a possible source of increased risk of bone fracture, especially in post-menopausal women.

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