Original Article:

Ultrasound bone mineral density of *Os Calcis* - its relationship with bone mineral markers and 25(OH) vitamin D in endemic fluorotic and non-fluorotic villages

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ABSTRACT

Objectives: To study the relationship between the nutritional status, serum bone mineral markers, 25 hydroxy vitamin D [25(OH)D] levels and ultrasound bone mineral density (USBMD) of *Os Calcis* in subjects living in endemic fluorotic and non-fluorotic villages.

Methods: Subjects from fluorotic (n=57) and non-fluorotic (n=79) villages were studied for their dietary habits, biochemical parameters of bone mineral markers, 25(OH)D levels and correlated with stiffness index (SI) measured using Achilles ultrasound bone densitometer.

Results: Dietary calcium intake in both the villages is far below the recommended daily allowances (RDA) by Indian Council of Medical Research (ICMR), India for Indian population. The 25(OH)D correlated positively with energy intake ($r=0.7$; $p<0.001$); dietary calcium ($r= 0.5$; $p<0.001$); and negatively with phytate/calcium ratio ($r=0.2$; $p<0.001$), in subjects in fluorotic villages. No similar correlation was observed among subjects from non-fluorotic villages. For comparable levels of serum calcium, subjects in non-fluorotic villages were more osteopenic than the fluorotic counterparts. USBMD did not correlate with 25(OH)D in the fluorotic and non-fluorotic subjects.

Conclusions: The dietary calcium intake among subjects from fluorotic and non-fluorotic villages is less than the RDA suggested by ICMR. The 25(OH)D levels of both these villages were in the vitamin D insufficiency range. USBMD does not correlate with the 25(OH)D status of an individual and it should not be used for screening osteoporosis in areas endemic for fluorosis.

Key Words: *Fluorosis, Vitamin D, Ultrasound bone densitometry, Os Calcis, Dietary calcium*

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INTRODUCTION

Caucasians and Asians are at the highest risk for involutional osteoporosis.[1] Quantitative ultrasound (QUS) of *Os Calcis* provides a precise, radiation free, low cost and rapid method of fracture risk assessment in clinical practice.[2] QUS has been found to be an independent predictor of osteoporosis fracture risk.[3] Measurements of bone mineral density (BMD) by ultrasound bone densitometer (USBMD) has been a predictor of hip fractures[2,4] and correlates with dual energy X-ray absorptiometry (DXA).[5,6] Measurement of stiffness index (SI) by Achilles ultrasound bone densitometer has been a predictor of fracture of spine and is comparable with the BMD measurement with standard DXA.[7,8] Advances in technology has considerably standardized the technique of QUS and improved the methodology and quality control of USBMD.[9-11] The drawback of techniques, problems in the precision of scanners, specificity and sensitivity of various parameters have all been overcome presently.

BMD measured by QUS has shown strong relationship with age, gender, body mass index (BMI), waist to hip ratio (WHR), smoking status, frequency of exercise etc.[1] There are studies[11-14] pointing to limitations of using calcaneal USBMD in screening, monitoring of therapy. Apart from the limitations intrinsic to

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the equipment, there are other extrinsic factors like chronic fluoride ingestion, bare foot walking, daily dietary calcium intake, vitamin D status of an individual, athletic build which can affect the USBMD measurements. So far there is no population study from India documenting the bone mineral parameters with USBMD in fluorotic and non-fluorotic villages. This publication tries to document these findings and limitations of USBMD in population screening.

MATERIAL AND METHODS

Between January 2000 and July 2003, 79 subjects from non-fluorotic villages [drinking water fluoride < 1 parts per million (ppm)] and 57 subjects from fluorotic villages (drinking water fluoride >2 ppm) were studied for their physical characteristics, dietary habits, bone mineral parameters and SI of *Os Calcis* by USBMD.

In both the villages dietary habits, the daily dietary intake of calcium, phosphorus and phytates were documented by recalling the diet consumed in the previous 5 to 7 days. From the raw weights, the calcium and phosphorous intakes were calculated using the published food composition table detailing the nutritive value of Indian foods.[15] The subjects were asked to remain fasting on the day of collection of blood sample. In both these locations, the average duration of sunlight is around 8 to10 hours per day, throughout the year. Winters are short with poor rainfall. Often there is little seasonal variation of the peak sunlight. The subjects are exposed to sunlight for a period of 8 to 10 hours a day. Their exposure to sunlight was not restricted by cloths or veil.

About 10 mL of blood was collected from the most accessible peripheral vein between 0700 to 0900 hours in fasting state without using a tourniquet for measurement of serum calcium, phosphorus, alkaline phosphatase (SAP), creatinine, and albumin concentrations. Blood was collected in test tubes kept under ice, for estimation of serum parathyroid hormone-intact molecule (Ntact-PTH) and 25 hydroxy vitamin D [25(OH)D]. Serum was separated in refrigerated centrifuge at 4 ^{O}C and stored at –20 ^{O}C until analysis. The blood samples collected from villages were transported under cool packs until they were separated and stored for further analysis. The methodology of evaluation of serum calcium, phosphorus, alkaline phosphatase (SAP), and albumin has been described in our previous publication.[16] The subjects were classified as vitamin D-deficient, insufficient, or sufficient on the basis of 25(OH)D concentrations of less than 20 ng/mL, 20-30 ng/mL, and greater than 30 ng/mL, respectively, according to recent consensus.[17-19] Normal values for these variables at our laboratory were as follows: serum calcium 8.5 - 11 mg/dL, serum phosphorus 2.5 - 4.8 mg/dL, SAP 40 - 90 IU/L and serum albumin 3 - 5.5 g/dL.

The 25(OH)D concentrations were measured by competitive radioimmunoassay (RIA) (DiaSorin, Stillwater, MN, USA, catalogue No. 68100E) and Ntact-PTH was measured by immunoradiometric assay (IRMA) (DiaSorin, Stillwater, MN, USA, catalogue No. 26100). The fluoride levels in water, serum and urine were measured by fluoride ion selective electrode (Orion electrode Model 740).[20] USBMD was measured by a portable ultrasound bone densitometer (GE healthcare - Achilles, USA) Using high frequency sound waves, the Achilles measures both the speed of sound (SOS) and broadband ultrasound attenuation (BUA). Achilles, analysis the density of the bone by measuring both how fast sound travels through the heel bone, and how much of the sound reaches the other side. Both measurements are combined into a single index called 'stiffness index' (SI). The SI is then used to create two separate scores, namely the T-score and Z-score. The SI is compared to reference figures for a healthy young adult to create the T-score. The Stiffness Index is compared to reference figures for a healthy adult in the patient's age group to create the Z-score. T-score indicates current risk for fracture as compared to a healthy young adult. Risk is defined as follows: a score higher than –1.0

indicates low risk; a score between –1.0 and –2.5 indicates intermediate risk and a score lower than –2.5 indicates a high risk.

STATISTICAL ANALYSIS

Descriptive results are presented as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was used to estimate the differences between the study groups. A p-value less than 0.05 was considered significant. Pearson's coefficient was calculated for the correlation. Probability values less than 0.05 were considered significant. Statistical analysis was performed using SPSS (version 11.5).

RESULTS

Fluorotic areas had water fluoride content greater than 2 PPM and non-fluorotic villages had water fluoride less than 1 PPM. The urine fluoride was significantly ($p<0.0001$) higher in subjects from fluorotic villages (2.11±0.08 PPM) compared with non-fluorotic villages (0.84±0.01 PPM). The mean age of subjects in the fluorotic and non-fluorotic areas was not significantly different. The subjects from fluorotic areas had higher BMI compared to subjects from non-fluorotic areas (Table 1). The daily dietary calcium intake of both the groups was far lower than the recommended daily/ dietary allowance (RDA) by Indian Council of Medical Research (ICMR) for Indian population. Yet the subjects in fluorotic areas had a significantly ($p<0.0001$) lower daily dietary calcium intake (242±3 mg/day) compared to their counter parts in non-fluorotic areas (282±2 mg/day).

Biochemically, subjects residing in fluorotic area had significantly ($p<0.0001$) lower serum

Table 1: Comparison of various parameters between patients from non-fluorotic and fluorotic areas

Parameter	Units	Patients from non-fluorotic areas (n =79)		Patients from fluorotic areas (n=57)	
		Mean	SEM	Mean	SEM
Water fluoride	ppm	< 1	NA	>2	NA
U.Fluor	ppm	0.84	0.01	2.11	0.08*
S.Fluor	ppm	0.05	0.00	0.13	0.00*
Age	years	48	1	45	2
BMI	kg/m^2	23.96	0.28	21.16	0.48*
D.Cal	(mg/day)	282	2	242	3*
D.Pho	(mg/day)	553	4.7	432	6*
S.Alb	(g/dL)	4.51	0.06	4.34	0.04†
S.Cal	(mg/dL)	10.1	0.07	9.67	0.08*
S.Phos	(mg/dL)	3.1	0.06	2.7	0.04*
SAP	(IU/L)	42.5	2	82.9	2.28*
25(OH)D	(ng/mL)	22.63	0.72	22.81	1.19
Ntact-PTH	(pg/mL)	23.47	0.77	37.93	0.80*
YA(SI)		76.44	2.36	84.16	2.44‡
T-score		–1.80	0.18	–1.29	0.21‡
AM(SI)		88.81	2.58	96.02	2.41‡
Z-score		–0.72	0.17	–0.25	0.17‡

*p <0.001; †p<0.01; ‡p<0.05

SEM = standard error of mean; U.Fluor = urine fluoride; S.Fluor = serum fluoride; D.Cal = dietary calcium; D.Phos = dietary phosphorous, S.Alb = serum albumin; S.Cal = serum calcium; S.Phos = serum phosphorus; SAP = serum alkaline phosphatase; 25(OH)D = 25 hydroxy vitamin D; Ntact-PTH = parathyroid hormone; YA(SI) = young adult (Stiffness Index); Ts = T-score; AM(SI) = age-matched (stiffness index); Zs = Z-score

calcium, phosphorus and elevated alkaline phosphatase compared to non-fluorotic subjects. The 25(OH)D levels were in the vitamin D insufficiency range and comparable in both the groups. Subjects residing in fluorotic area had a significantly ($p<0.0001$) higher PTH compared to subjects residing in non-fluorotic area (38±0.8 Vs 23.5±0.8 pg/ml respectively) though both were within the normal range of the kit manufacturer. Residents of fluorotic area had a significantly higher USBMD parameters compared to those residing in non-fluorotic area.

In residents of fluorotic area, urinary fluoride correlated positively with serum fluoride ($r = 0.8$; $p<0.0001$). Age correlated positively with urine fluoride ($r = 0.6$; $p<0.0001$) and serum fluoride ($r = 0.7$; $p<0.0001$). T-score correlated negatively with serum fluoride ($r = –0.33$; $p<0.01$) and urine fluoride ($r = 0.3$; $p<0.03$). Age correlated negatively with T-score ($r = –0.5$; $p<0.001$) and Z-score ($r = –0.3$; $p<0.02$). In residents of non-fluorotic area, there was no correlation of serum or urine fluoride with age, T-score or Z-score. But, there was a negative correlation of age with T-score ($r = –0.4$; $p<0.001$) and Z-score ($r = –0.4$; $p<0.002$).

Dietary calcium correlated positively with serum fluoride ($r = 0.3$; $p<0.02$), urine fluoride ($r = 0.4$; $p<0.001$) and 25(OH)D levels ($r = 0.5$; $p<0.001$) in subjects residing in fluorotic areas. In residents of non-fluorotic areas dietary calcium correlated positively with serum fluoride ($r = 0.3$; $p<0.01$) and urinary fluoride ($r = 0.35$; $p<0.002$). In subjects residing in non-fluorotic area dietary calcium correlated positively with T-score ($r = 0.3$; $p<0.01$) and Z-score ($r = 0.24$; $p<0.03$). No such correlations were evident in subjects residing in fluorotic area.

In subjects residing in fluorotic area, SAP correlated positively with serum fluoride and urine fluoride ($r = 0.3$; $p<0.02$). Serum phosphorus correlated negatively with serum fluoride ($r = –0.33$; $p<0.001$) and positively with T-score ($r = 0.34$; $p<0.01$). No such observations were seen in subjects residing in non-fluorotic areas. There was strong internal correlation between T-score and Z-score ($r=0.09$; $p<0.001$) in subjects residing in fluorotic and non-fluorotic areas.

In the present study, subjects residing in fluorotic areas had a higher serum and urine fluoride compared with those residing in non-flurotic areas. As a group, the subjects residing in fluorotic areas had lower dietary calcium, serum calcium and phosphorous compared to the subjects in non-fluorotic counterparts. They had high SAP, PTH and comparable levels of 25(OH)D compared to the subjects residing in non-fluorotic areas. The USBMD parameters in subjects with fluorotic areas were significantly high compared with the subjects residing in non-fluorotic areas.

DISCUSSION

Assessment of bone by QUS is a strong predictor of hip fractures. It is a United States Food and Drug Administration (US-FDA) approved tool to identify women at risk of osteoporosis.[21] It correlates moderately with DXA and hypovitaminosis D.[22] There is not much data available on the limitation of using USBMD in the evaluation of subjects with osteoporosis in endemic fluorotic areas. Fluoride is know to increase the bone density but with a poor crystalline matrix. It mobilizes mineral from the axial skeleton and distributes it to the appendicular skeleton.[23] The bone appears dense due to high fluoride content but does not reflect the strength. Because of the redistribution of the mineral, a measurement of peripheral bone density may not truly correlate with the spine or femoral hip bone mineral density. These factors limit the use of USBMD in screening for osteoporosis in residents of endemic fluorosis.

Chronic dietary calcium insufficiency adversely affect bone mineral metabolism. The long standing secondary hyperparathyroidism (SHPT) leads to osteopenia and increases the risk of fractures.[24,25] High phytate calcium ratio reduces the calcium absorption from the gut. There are reports of low serum calcium with

raised PTH in patients with endemic skeletal fluorosis.[26] Chronic SHPT due to dietary calcium insufficiency and fluorosis leads to increased risk of fractures. The combination of dietary calcium insufficiency, high phytate calcium ratio and environmental toxin fluoride over a long period from child hood can reduce the peak bone mass with propinquity to fractures in adult life. The residents of fluorotic areas had chronic fluoride toxicity as evidenced by high fluoride content in their drinking water, diet along with low dietary calcium and high phytate calcium ratio. The effects of these changes were reflected as high SAP and PTH in this group of patients. The comparable levels of 25(OH)D in both the groups can be attributed to their dress code and agricultural occupation.

The calculated dietary calcium was far less than the RDA suggested by ICMR in subjects residing in the fluorotic and non fluorotic areas. It has been shown in the studies that the calculated values for all nutrients are significantly higher than the analytical values.[27] There are reports of low dietary calcium intake along with low 25(OH)D levels in South Indian population.[28,29] There are studies to show dietary calcium supplementation reduces the risk of fractures in elderly.[25]

The USBMD parameters in residents of non-fluorotic areas were less compared to residents of fluorotic areas (Table 1). Low dietary calcium positively correlated with USBMD parameters in residents of non-fluorotic areas. In residents of fluorotic areas no such correlation was found. This is probably because fluoride alters the crystalline structure of bone and increases the bone density. The dense bone alters the SOS and BUA depicted as higher T-scores and Z-scores. This is seen as a negative correlation between age, serum and urine fluoride with T- and Z-scores.

Achilles measures the bone density of the heel using high frequency ultrasound waves. Achilles measures both the SOS and BUA. It analyses the density of the bone, by measuring both how fast sound travels through it, and how much of the sound reaches the other side. Achilles uses water as an ideal coupling solution and it is thermally controlled to 92 °C to obtain the best possible results.

In a study[30] of 304 postmenopausal women (age 58.8 ± 5.5 years) BMD of the lumbar spine (by dual-energy X-ray absorptiometry), SOS, BUA and stiffness in the Os Calcis (using an Achilles machine) were measured. In the whole population SOS, BUA and stiffness values were significantly correlated with BMD. SOS, BUA and stiffness values were significantly decreased ($p<0.001$) with vertebral deformity, as was BMD. Logistic regression analysis showed that BMD, BUA, SOS and stiffness were independent predictors of vertebral fracture risk. Ultrasound parameters were still significant independent predictors of vertebral fracture even after adjusting for BMD.[30] In another study,[31] it was shown that the SI was a significantly better indicator than BUA or SOS in identifying post-menopausal women with low BMD and/or osteoporotic fractures.

The present study brings forth the following points. The dietary calcium intakes of both the fluorotic and non-fluorotic villages is less than the RDA suggested by ICMR. The 25(OH)D levels of both these villages were in the vitamin D insufficiency range. USBMD does not correlate with the 25(OH)D status of an individual. Fluoride forms fluroappatite in the bone which is hyper dense and can fallaciously produce increased bone density. Dietary calcium supplementation and supplying water free from fluoride benefit these patients. USBMD should not be used for screening osteoporosis in areas endemic for fluorosis.

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