

DUAL ENERGY X-RAY ABSORPTIOMETRY (DXA) STUDY OF ENDEMIC SKELETAL FLUOROSIS IN A VILLAGE OF NALGONDA DISTRICT, ANDHRA PRADESH, INDIA

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SUMMARY: A comparative study of bone mineral density (BMD) and bone fracture was conducted in a fluorotic and a nonfluorotic area of the Nalgonda District, Andhra Pradesh, India. BMD measured by dual X-ray absorptiometry (DXA) of L2-L4 vertebrae, femoral neck, hip, and whole body was significantly higher by 112%, 43%, 57%, and 50%, respectively, in 12 fluorotic subjects than in 14 nonfluorotic subjects ($p < 0.01$). However, there was an 11% decrease, although not statistically significant, in forearm BMD in the fluorotic subjects compared to the nonfluorotic subjects. Serum levels of total and bone specific alkaline phosphatase in the fluorotic subjects were significantly elevated by 219 and 313%, respectively ($p < 0.01$), whereas serum Ca, protein, and phosphorus were 10, 12, and 32% lower, respectively ($p < 0.01$). On the other hand, serum creatinine, urea, and zinc levels in the two groups of subjects were not significantly different. In the fluorotic village, with a 34% lower average consumption unit intake of calcium, the overall bone fracture rate of 6.3% was significantly higher than the 2.1% rate in the nonfluorotic village ($p < 0.05$).

Keywords: Bone fracture; Bone mineral density; Dietary calcium; Dual energy X-ray absorptiometry; Endemic fluoride area; Skeletal fluorosis.

INTRODUCTION

Fluoride (F) is toxic to osteoblasts (bone forming cells) and resorbing osteocytes.¹ A first response to cell injury is to initiate repair, and when that fails the cell dies. In F intoxication the repair mechanism fails, and the result is an initial increase in both bone formation and resorption. This response to cell injury by F leads to pathological bone formation, and such increased formation or decreased resorption of bone results in increased bone mass.² When injured osteoclasts die, new osteoclasts are formed from monocytes. Thus secondary injury of osteoclasts does not result in a paucity of osteoclasts on the surface of fluorotic bone.³ F injures cells involved in bone formation, and a poorer quality bone accumulates in patients with increased intake of F.⁴ However, the newly formed bone is inferior, and the matrix is irregular.^{3,5-7} Its collagen structure also differs from that associated with normal bone,⁶ and its mineralization is enhanced.^{3,5-10} Although F increases bone mass or bone mineral density (BMD), the newly formed bone may have reduced strength, since F increases widening of osteoid seams and creates cytological and matrix abnormalities. Increased brittleness and fragility due to increase in osteocyte resorption leads to an increase in the number of micro fractures in bone.¹¹⁻¹³ This has been proved in a field study¹⁴ showing that higher F intake increases bone fracture incidence, and it did not benefit vertebral fracture risk.

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Hydrofluorosis is a major public health problem in India, affecting 18 of the 33 constituent states and Union territories. About 62 million people including 6 million children are estimated to suffer from various degrees of fluorosis, including skeletal manifestations due to excessive consumption of F through drinking water.¹⁵ Under these circumstances there is a need for a sensitive method to diagnose skeletal fluorosis in the Indian context to overcome confusion about high BMD and bone health in the elderly and women of postmenopausal age.

However, as far as we are aware, no study to meet this need has been undertaken with appropriate dual energy X-ray absorptiometry (DXA) scanning along with an assessment of calcium intake, bone fracture rate, and kidney status in an endemic fluorosis area for comparison with a nonfluorotic area. Here an attempt has been made in this direction.

SUBJECTS AND METHODS

For this study, the four villages of Chinnanaryanpur, Godukundla, Yedavally, and Lanchammagadam in the Nalgonda district of Andhra Pradesh were randomly selected for their presence in an endemic fluorosis area. Analysis of their drinking water indicated that the F levels were 2.1, 3.1, 4.5, and 4.5 ppm, respectively. Yedavally village and Addagutta, an urban slum, were randomly selected as the fluorotic (4.5 ppm F) and nonfluorotic (F<1.5 ppm) areas, respectively, for the study.

Calcium (Ca) intake by the 24-hr recall method, height and weight by standard procedure, and history of bone fracture were determined by interview and measurement. Clinical examination for dental mottling, skeletal deformities (genu valgum, genu varum, bowing of tibia, kyphosis, exostosis, scoliosis, muscular tenderness, neck rigidity, and stiffness of joints) was done among the entire population of the fluorotic Yedavally village as well as the nonfluorotic Addagutta slum by a well-trained technician.

Subjects: The initial study population consisted of 250 subjects from 55 households of Yedavally and 250 subjects from 50 households of Addagutta. Owing to the expensive nature of DXA testing, only 12 (six postmenopausal women and six men with a mean age of 53.21 years) from the fluorotic village of Yedavally and 14 age and sex matched subjects from the nonfluorotic control village of Addagutta were randomly selected for DXA scanning and various biochemical determinations.

Biochemical analysis: Water samples were collected from all drinking water sources of both villages in plastic bottles. Spot urine samples were also collected in plastic bottles using toluene as a preservative from a sub-sample of the randomly selected subjects. From those selected for DXA scanning, fasting blood samples were collected in plain glass test tubes, serum was separated, and total alkaline phosphatase and bone specific alkaline phosphatase were analyzed on same day by heat inactivation method of Klaus Walter and Moss method.^{16,17} The rest of the serum samples were preserved at -75°C for further analysis.

The F content of water, urine, and ionic F in serum were determined with an Orion F ion specific electrode (EA 940, Boston, MA, USA). Serum protein was measured by the biuret method, Ca and zinc by atomic absorption spectrophotometry (Spectra AA-220, Varian, India), phosphorus by the procedure of Fiske and Subba Row,¹⁸ and urea and creatinine by standard methods.^{19,20}

Food and nutrient intake: In order to assess calcium intake, a semiquantitative diet survey using the 24-hr recall method²¹ was carried out among the 10% of the households in each of the two villages. The intakes of different foods were converted to nutrients per consumption unit (CU) by using food composition tables.²²

X-ray: X-rays of the left arm was taken to confirm the presence of fluorosis.

Bone mineral density measurement: Bone mineral density (BMD) (g/cm²) measurements were carried out by dual energy X-ray absorptiometry (DXA) using Hologic 4500w Waltham, MA bone densitometry equipment. BMD was measured at three sites, i.e., anteroposterior (AP) lumbar spine (L2–L4) (LS), hip including femoral neck (FN), forearm, and the whole body (WB), by a trained person who carried out all the scans and analyzed the results according to the manufacturer's instructions. The numbers of measurements were 7 and 10 for forearm, 14 and 12 for whole body, and 14 and 11 for hip in control and fluorotic subjects, respectively. Measurements on forearm could not be done on all subjects because some women had metal jewelry, which could not be removed and would interfere with the measurements. The scanner was calibrated daily, using a control phantom, and its performance was monitored as per the quality assurance protocol. No sign of scanner drift was observed during the study period. The *in vivo* precision (coefficient of variation) was 1–2% for all the bone density measurement.

Bone fracture history: By use of the same questionnaire, uniform information on bone fracture history was collected on 238 individuals in the fluorotic area and 236 in the nonfluorotic area. The information collected included site and year of fracture for persons below age 70.

Statistical Analysis: Descriptive statistics are reported for all variables. Student's t test was performed for comparison of mean values between groups of fluorotic and nonfluorotic subjects. The Mann-Whitney U test was applied for comparison of mean values when the assumption of homogeneity of variances was violated. Prevalence of bone fractures were compared by chi-square test with one degree of freedom. SPSS windows version 11.5 was used for statistical analysis. $P < 0.05$ was considered significant.

RESULTS

Except for drinking water and urinary F, the differences in age, weight, height, total fat, and calcium consumption units in the fluorotic and nonfluorotic subjects were not statistically significant (Table 1). Fluorosis in fluorotic subjects was confirmed by forearm X-ray, in some cases showing intraosseous membrane calcification (Figure 1) not due to bone fracture. Knee deformities like genu

valgum and genu varum (Figure 2) were found in 4% and 6% of the population, respectively, along with 0.5% paraplegia, in the fluorotic area, while no such cases were seen in the control area. The 6.3% rate of bone fracture in the fluorotic area was significantly higher than the 2.1% rate in the nonfluorotic area ($p < 0.05$) (Figure 3).

Table 1. Drinking water and urinary F plus anthropometric parameters (mean±SD)

Group	Drinking water fluoride (ppm)	Urinary fluoride (ppm)	Age (yrs)	Weight (kg)	Height (cm)	Total fat (%)	Calcium intake (mg/CU) ^a
Nonfluorotic (n=14)	1.0 ±0.3	1.2 ±0.4	53.2 ±10.26	52.0 ± 9.40	151.7 ± 7.21	30.6 ± 9.15	181.5 ± 63.16
Fluorotic (n=12)	4.5 ± 1.6*	7.6 ± 1.5*	53.3 ±11.08	47.8 ± 10.17	153.1 ± 6.14	24.3 ± 0.73	119.8 ± 58.10

^aCU = Consumption Unit (defined in terms of mg Ca intake by a 60-kg middle-age male per 2425 kcal food energy intake/day). * $p < 0.05$.



Interosseous membrane calcification

Figure 1. X-ray of forearm from fluorotic (right, age 54yr) and nonfluorotic (left, age 51yr) village.

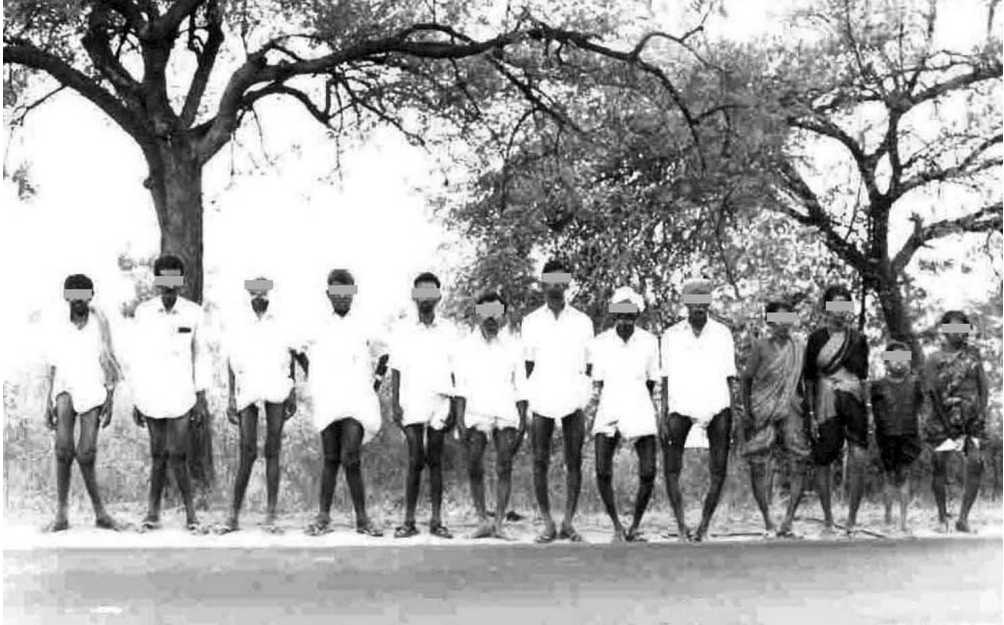


Figure 2. Photograph showing subjects with skeletal deformities from fluorotic Yedavally village.

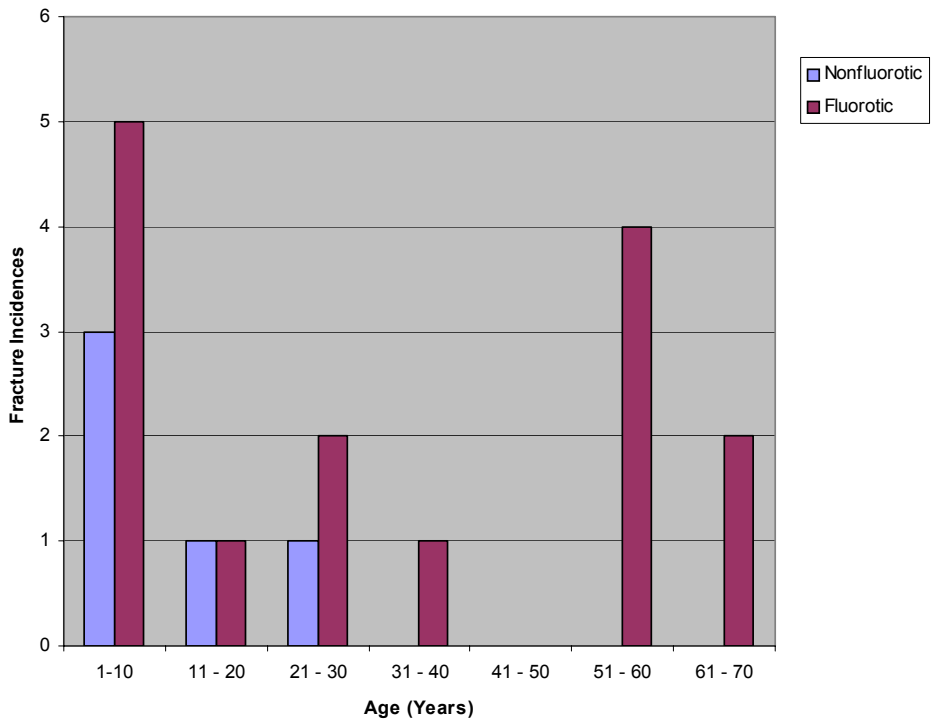


Figure 3. Fracture incidences in fluorotic Yedavally and nonfluorotic Addagutta villages.

Serum levels of ionic F and of total and bone specific alkaline phosphatase were significantly higher in the fluorotic subjects, whereas serum levels of protein, Ca, and phosphorus were significantly lower. However, there were no significant differences in the levels of serum creatinine, urea, and zinc (Table 2).

Table 2. Serum biochemistry in fluorotic and control subjects (mean±SD)

Group	Protein (g/dL)	Ca (mg/dL)	P (mg/dL)	Zn (µg/dL)	SAP ^a (IU/L)	BspAP ^b (IU/L)	Ionic F (ppm)	Creatinine (mg/dL)	Urea (mg/dL)
Nonfluorotic (n=8)	8.5 ± 0.76	10.2 ± 1.09	3.6 ± 0.71	92.4 ± 21.37	49.7 ± 16.87	27.9 ± 14.37	0.02 ± 0.01	1.1 ± 0.17	35.8 ± 4.82
Fluorotic (n=11)	6.7 ±1.50*	9.2 ±0.70*	2.4 ±0.36*	85.8 ±15.12	158.4 ±68.62*	115.4 ±79.52*	0.21 ±0.11*	1.0 ± 0.14	42.3 ± 18.43

^aSAP = serum alkaline phosphatase; ^bBspAP = bone specific alkaline phosphatase. *p<0.05.

As can be calculated from Table 1, the average dietary consumption unit intake of Ca in the fluorotic group was 34% lower than in the nonfluorotic group; however, the difference is not statistically significant because of wide variations.

Bone density parameters: As seen in Table 3, the BMD of L2–L4 vertebrae, femoral neck, hip, and whole body was significantly higher in the fluorotic than in the nonfluorotic subjects (p<0.01). However, there was a small decrease in forearm BMD in the fluorotic subjects that was not statistically significant.

Table 3. Bone mineral density (gm/cm²) (mean ± SD)

Bone parameter BMD	Nonfluorotic group	Fluorotic group	Mean percent higher BMD of fluorotic vs. nonfluorotic group
Femoral neck	0.7 ± 0.11 (n = 14)	1.0 ± 0.18* (n = 11)	43
Hip	0.7 ± 0.12 (n = 14)	1.1 ± 0.16* (n = 11)	57
Lumbar spine	0.8 ± 0.13 (n = 14)	1.7 ± 0.430* (n = 12)	112
Forearm	0.5 ± 0.09	0.45 ± 0.09*	-11
Whole body	1.0 ± 0.28 (n = 14)	1.5 ± 0.24* (n = 12)	50

*p<0.001.

DISCUSSION

Lumbar spine (predominantly cancellous bone) BMD was 112% higher in the fluorotic group than in the nonfluorotic group, in agreement with results of F therapy research.²³ However, there was a decrease in BMD the forearm radius, a site with predominance of cortical bone. Such findings have been reported during F treatment.^{24–27} These results suggest that F ingestion causes a redistribution of bone from cortical to cancellous compartments. Here this may be because of a low dietary calcium intake (normally found in rural Indian populations), which cannot cope with the large amount of newly formed matrices in cancellous bone, resulting in the removal of mineral from cortical bone by increasing in iPTH.²⁸ Fluoride injures all the cells in bone and alkaline phosphatase is released with a rise in serum alkaline phosphatase (SAP). The SAP increase observed in the present study was inversely correlated with a small lowering of serum Ca. This increase in SAP is consistent with the increased bone formation and decreased bone turnover

that results from the death of activated, resorbing osteocytes. SAP in nutritional secondary hyperparathyroidism reflects the fact that alkaline phosphatase is also an enzyme of bone resorption.

Results of the present investigation agree with earlier F therapy studies^{23,24} showing a smaller increase in femoral neck BMD as compared to the lumbar spine. We also found a higher BMD in the lumbar spine and femur neck in fluorotic subjects as compared to nonfluorotic subjects, and this may be due to prolonged intake of high F (known for amorphous bone formation) from drinking water and poor Ca nutrition. It is well known that poor nutrition and low Ca intake enhance the deleterious effect of fluoride.²⁷

As already noted, incidences of bone fracture (mainly Colley fracture of the forearm bone near the wrist and the proximal femur) were significantly greater in the fluorotic village of Yedavally than in the nonfluorotic control village of Addagutta. Thus, although F increased bone quantity (BMD), it did not improve bone quality and strength, in agreement with findings in an earlier field study in the city of Durango and surroundings in northwestern Mexico.¹⁴ In that study a linear correlation between the Dean index of dental fluorosis and frequency of bone fractures among children and adults was observed. A similar study conducted in China¹⁵ also revealed a higher prevalence of hip fractures in subjects with high levels of F (4.32–7.97 ppm) in their drinking water. It is also known that bone formed in response to large doses of F has increased crystallinity and thus may have decreased elasticity and greater susceptibility to stress fractures,^{28,29} in agreement with our findings of increased fractures in the fluorotic area.

In regard to effects on kidney function, the serum urea level was higher in the fluorotic subjects but not significantly so, and the creatinine level was virtually unchanged from that of the nonfluorotic control subjects. Thus the kidney status did not seem to be especially affected.

In conclusion, high F and low Ca intake were found by DXA to be associated with elevated BMD in cancellous bone, evidently leading to greater susceptibility to bone fracture. Also important is the small increase in BMD at sites composed of mixed cortical and cancellous bone along with a decrease in BMD at the sites containing predominantly cortical bone.

REFERENCES

- 1 Krook L, Minor RR. Fluoride and alkaline phosphatase. *Fluoride* 1998;31:177-82.
- 2 Dambacher MA, Lauffenberger T, Lammle B, Haas HG. Long term effects of sodium fluoride on osteoporosis. In: Courvoiser B, Donath A, Baud CA, editors. *Fluoride and bone*. Proceedings of the 2nd Symposium CEMA; Bern: Hans Huber; 1978. p. 238-9.
- 3 Krook L, Maylin GA. Industrial fluoride pollution. Chronic fluoride poisoning in Cornwall Island cattle. *Cornell Vet* 1979;69(Suppl 8):S1-70.
- 4 Vigorita VJ, Suda MK. The microscopic morphology of fluoride-induced bone. *Clin Orthop Relat Res* 1983;177:274-82.
- 5 Shupe JL, Miner ML, Greenwood DA. Clinical and pathological aspects of fluorine toxicosis in cattle. *Ann NY Acad Sci* 1964;111:618-37.
- 6 Franke J, Rath F, Runge H, Fengler F, Auermann E, Lenart G, et al. Industrial fluorosis. *Fluoride* 1975;8(2):61-83.

- 7 Johnson LC. Histogenesis and mechanism in the development of osteofluorosis. In: Simons JH, editor. Fluorine chemistry. vol. IV. New York and London: Academic Press; 1965. p. 424-41.
- 8 Stutzmann J, Petrovic A. Rate of remodeling of adult alveolar bone before and during orthodontic treatment. *Rev Orthop Dento Faciale* 1980;14(4):437-56.
- 9 Malcolm AS, Storey E. Osteofluorosis in rabbit: microradiographic studies. *Pathology* 1971;3:39-51.
- 10 Bernstein DS, Cohen P. Use of sodium fluoride in the treatment of osteoporosis. *J Clin Endocrinol Metab* 1967;27:197-10.
- 11 Baylink DJ, Bernstein DS. The effects of fluoride therapy on metabolic bone disease. *Clin Orthop Relat Res* 1967;55:51-85
- 12 Hedlund LR, Gallagher JC. Increased incidence of hip fracture in osteoporotic women treated with sodium fluoride. *J Bone Miner Res* 1989;4(2):223-5.
- 13 Herrera MTA, Dominguez IRM, Vazquez RT, Dozal SR. Well water fluoride, dental fluorosis, and bone fractures in the Guadiana valley of Mexico. *Fluoride* 2001;34:139-49.
- 14 Liang C, Ji R, Cao J, Cheng X. Research on the relationship between populations long term exposure to fluoride in drinking water and bone fracture in China. *Wei Sheng Yan Jiu* 2001;30:287-93.
- 15 Susheela AK. Fluorosis management programme in India. *Curr Sci* 1999;77:1250-6.
- 16 Klaus W, Schutt C. In: Bergmeyer HU, editor. Methods of enzymatic analysis. vol. II. New York: Academic Press; 1971. p. 856.
- 17 Moss DW, Whitby LG. Simplified heat inactivation method for investigation alkaline phosphatase isoenzymes. *Clin Chim Acta* 1975;61(1):63-71.
- 18 Fiske CH, Subba Row Y. The colorimetric determination of phosphorus. *J Biol Chem* 1925;66:375-8.
- 19 Wybenga DR, Di Giorgio J, Pileggi VJ. Manual and automated methods for urea nitrogen measurement in whole serum. *Clin Chem* 1971;17:891-5.
- 20 Yatzidis H. New method for direct determination of "true" creatinine. *Clin Chem* 1974;20:1131-4.
- 21 Hu FB, Rimm E, Smith-Warner SA, Feskanich D, Stampfer MJ, Ascherio A, Sampson L, Willett WC. Reproducibility and validity of dietary patterns assessed with a food frequency questionnaire. *Am J Clin Nutr* 1999;69:243-9.
- 22 Gopalan C, Ramshastry BV, Balasubramanyam SC, Narasinga Rao BS, Deosthale YG, Pant KC. Hyderabad, India: Nutritive value of Indian Foods, National Institute of Nutrition (NIN), Indian Council of Medical Research (ICMR);1987 and 1989.
- 23 Riggs BL, Hodgson SF, O'Fallon WM, Chao EY, Wahner HW, Muhs JM, et al. Effect of fluoride treatment on the fracture rate in postmenopausal women with osteoporosis. *N Engl J Med* 1990;322:802-9.
- 24 Riggs BL. Treatment of osteoporosis with sodium fluoride: an appraisal. In: Peck WA, editor. Bone and Mineral Research Annual, Vol. 2. New York: Elsevier; 1984. p. 366-93.
- 25 Ruegsegger P, Ruegsegger E, Ihner J, Dambacher M. Natural course of osteoporosis and fluoride therapy—a longitudinal study using quantitative computed tomography. *J Comput Asst Tomogr* 1985;9:626-7.
- 26 Krishnamachari KAVR. Skeletal fluorosis in humans: a review of recent progress in the understanding of the disease. *Prog Food Nutr Sci* 1986;10:279-314.
- 27 Mithal A, Trivedi N, Gupta SK, Kumar S, Gupta RK. Radiological spectrum of endemic fluorosis: relationship with calcium intake. *Skeletal Radiol* 1993; 22:257-61.
- 28 Eanes ED, Reddi AH. The effect of fluoride on bone mineral apatite. *Metab Bone Dis Res* 1979;2:3-10.
- 29 Wolinsky I, Simkin A, Guggenheim K. Effect of fluoride on metabolism and mechanical properties of rat bone. *Am J Physiol* 1972;223:46-50.