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Research Article

Biophysical and Biochemical Markers of Skeletal Fluorosis

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Abstract: Fluoride toxicity is a burgeoning problem in worldwide and also in Rajasthan, India. It may be serious problems in health of the adults and children. Several clinical and experimental studies have reported that the F induces bone deformities in skeletal fluorosis but mechanism of action is still unknown. In the present study, 55 male flurotic patients (age 33.9 ±8.1) were selected from the orthopaedic outdoor patient department at Nims Medical Hospital Jaipur India. The age matched controls (34.5±7.6) were selected from the area where fluoride content was less than 1.5 ppm. Tibial bowing, Saber Shin, bow-legs, Genu valgum, wider ends of long bones with other typical skeletal deformities and bone mineral density were examined to compare subjects and controls along with changes at diaphysis and metaphyses with calcification and ossification of ligaments. The serum biochemical marker namely alkaline phosphatase (ALP), hydroxyproline, vitamin D, calcium and phosphate were also measured. Results of the present study demonstrate presence of high phosphorus, ALP, hydroxyproline and reduced concentration of 25 Hydroxy Vitamin D and calcium among the fluorotic patients. On the basis of results it may conclude that different biochemical parameters provides a reliable indicator for monitoring the health status of the high endemic area of fluoride which are at risk of fluorosis.
Keywords: Fluorosis, Biochemical Markers, Bone deformities

INTRODUCTION

Ground water is one of the most important sources of drinking water and fluoride contamination in ground water is increasingly becoming a matter of great concern. The recommended concentration range in the drinking water is > 1.5ppm [1]. An estimated 66.6 million people (17 states in India) are at risk of acquiring Fluorosis. In Rajasthan, people of 22 districts (out of 32) are presently consuming fluoride [3-4] greater than permissible limit. Almost all districts in Rajasthan have fluoride greater than the permissible limit. This may be upto 18.0 ppm[2] . Various researches have been conducted to develop biomarkers of early fluorosis in animals. Interactions between fluoride and free-radical reactions have been studied in various biological systems including fluorosis[3.] Fluoride is cumulative toxin which can change accrual & resorption of bone tissue. It is able to alter the internal environment of bone [4]. Acute or chronic exposure to flouoride can lead to various changes in bone. Recently [5], pointed out that Fluoride uptake occurs in different stages. (a) It migrates into the hydration shells of bone crystallite. (b) Fluoride association with or incorporation into precursors of hydroxyl fluoroapatite and (c) Apatitic fluoride re-enters the circulating body fluids as a result of the long term process of bone resorption [6]. Fluorosis is a slow and progressive process causing symptoms related to several other systems particularly related to musculo-skeletal system [7]. Clinical symptom includes leading to crippling life of a person. Person may exhibit low back pain, joint pains, myalgia and other general features. Person may suffer from various deformities of limb as well as of axial skeleton [8].

Keeping in view the paucity of information in relation to fluoride and its causing bone deformities in population residing in fluoride endemic areas and its impact on health and society, the present study was undertaken. The significance of this study is to investigate the biochemical and biophysical markers for the detection of Fluorosis.

METHODOLOGY

Subject selection

In the present study, 55 male fluorosis patients (age 32 ± 3.1) were selected after the performance of Chin Chan test (WHO) and serum fluoride levels in subjects. The age matched controls were selected from the area where fluoride content was less than 1.5 ppm.

Biophysical Assessment

Tibial bowing, genu varum, Genu valgum, wider ends of long bones with other typical skeletal

deformities and bone mineral density were examined to compare subjects and controls along with changes at diaphysis and metaphyses with calcification and ossification of ligaments.The BMD estimation was done using hologic QDR bone densitometer machine which uses pencil beam technology to assess BMD [9].

All subjects had gone through history,complete clinical examination and physical assessment of other body systems. Genu valgum and genu varum which is one of biophysical markers were assessed using intermalleolar distances as well as as intercondylar distances in standing position. Subjects who were having intermalleolar distances more than 10 cm were sent for radiological examination [10]. Radiological assessment was done independently by department of radiology; NIMS medical college. They examined the subjects by having x rays of extremities & spine. Subjects and controls were assessed for changes at the distal ends of long bones,presence of ossification of ligaments, presence of exostosis and deformity.

Biochemical Assessment

After clinical examination of subjects and controls, 5.0 ml of blood sample was drawn under complete aseptic condition in simple vial and was allowed to clot at room temperature. The separated serum was used to measure serum fluoride levels using specific fluoride electrode (Thermo Fischer, Singapore) and biochemical investigations. The plasma baseline 25(OH) vitamin D levels were measured by high performance liquid Chromatography. Total calcium, phosphate, alkaline phosphate and hydroxy proline determined by chemiluniscence method in routinely laboratory test.

RESULTS

There was no statistically significant difference between age, gender and BMI of the two groups (Table 1). The concentration of fluoride was found to be more significantly elevated in the serum of subjects. The biophysical property were exhibited bone deformities (Table 2) in term of of ossification or calcification of ligamentous attachments. Ossification of the inteosseous membrane in the forearm (Fig. 2) typically appeared first on the radial side and occasionally found first on the ulna side. The fused radius and the ulna were also found by ossified interosseous membranes. On the other hand other features (broadening of ends of long bones) were observed in about 28% of subjects (Table 2). In our study genu valgum is found in about 36.36% of cases while genu varum was found in about 9.09% of cases [11-14]. The anterior bowing of tibia was present only in 7.2 % of cases. No patient present with severe manifestation like exostosis. Kyphotic deformity was also not found, which was most probably due to fact that our subjects were not beyond 50year of age. In our study only 19% of patients were not having any deformity. These patients were most probably migrated to fluoride area in their late adolescence. In our study we did measured BMD at femur neck, wards triangle at proximal femur, femur trochanter & lumber 2-4 vertebrae of spine (Table 3). These parts consist of good amount of cancellous bone, where we can detect changes in flouorosis.In this study subjects have higher amount of BMD in comparison to control person. The significant p value is noted at trochanter femur and at spine. Even with higher BMD one of patient present with intertrochanteric fracture (Fig. 2) [15]. The biochemical investigations are presented in table 4. The concentration of ALP were found to be significantly ($P<0.001$) increased in subjects as compared with the controls. The level of Calcium and phosphorous were eleveted ($P<0.01$) while, 25 OHD was decresed in the serum of subject when compared with the controls. The concentration of hydroxy proline were found to incresead markedly ($P<0.01$) in subject than that of controls.

Table 1: Demographic data of Subjects and controls

	Control (55)	**Exposed (50)**
Age	34.5±7.6	33.9 ±8.1
BMI	22.7 ± 1.2	21.2 ± 1.0
Socio-economic status	Lower (100%)	Lower (100%)
Literacy (H.Sc.)	100 %	100 %
Smokers	58%	51%
Alcoholic	4% (occasionally)	3.5% (occasionally)

Age and BMI are expressed as mean ± SD and others parameters represented in percentage in control and subjects.

Table 2: Biophysical markers of skeletal fluorosis

Deformity	**No. of subjects (n=55)**	**Total percentage**	**No. of controls (n= 55)**	**Total percentage (n1) %**
Genu valgum	20	36.36	0	0
Genu Varum	5	9.09	0	0
Anterior bowing of tibia	4	7.2	0	0
Other features (changes at distal ends of long bones, presence of exostosis)	15	27.27	0	0
No deformity	10	18.18	55	100
Total	55	100%	55	100%

Data are expressed as total percentage (%) in subject and control.

Table 3: Bone mineral Density (BMD) in Subjects and controls

	Z score			T -score		
	Control	**Subjects**	**p-value**	**Control**	**Subjects**	**p-value**
Femur Neck	0.79 ± 0.82	2.49 ± 1.1	P<0.001	0.16 ± 1.0	1.81 ± 1.1	P<0.01
Femur Wards	0.82 ± 0.86	1.52 ± 1.09	P<0.05	0.23 ± 0.98	3.85 ± 1.1	P<0.001
Femur Trouchanter	0.84 ± 0.81	3.55 ± 0.94	P<0.001	0.43 ± 0.78	1.94 ± 1.12	P<0.001
Spine BMD	0.91 ± 0.71	2.54 ± 1.34	P<0.001	0.31 ± 0.87	2.01 ± 1.07	P<0.001

Data are expressed as mean ± SD in control and subjects. Significant between groups was determined by Mann-Whitney *p*-test

Table 4: Biochemical markers in Subjects and controls

	Control (55)	**Exposed (55)**	**P value**
ALP (IU/L)	74.5±2.3	89.7±4.2	P<0.001
25 OHD (ng/ml)	39.7±3.1	23.2±4.78	P<0.05
Calcium (mmol/L)	2.35±0.2	2.68±0.2	P<0.01
Phosphorous (mmol/L)	1.02±0.13	1.39±0.18	P<0.01
Hydroxy proline (mg/L)	14.1±2.2	16.2±2.8	P<0.01

Data are expressed as mean ± SD in control and subjects. Significant between groups was determined by Mann-Whitney *p*-test.

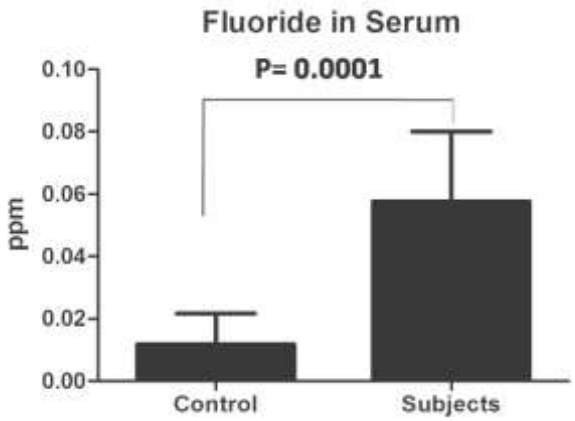

Fig. 1: Serum Concentration of fluoride (ppm) in subjects and control. The results are expressed as mean ± SD for control and subjects.

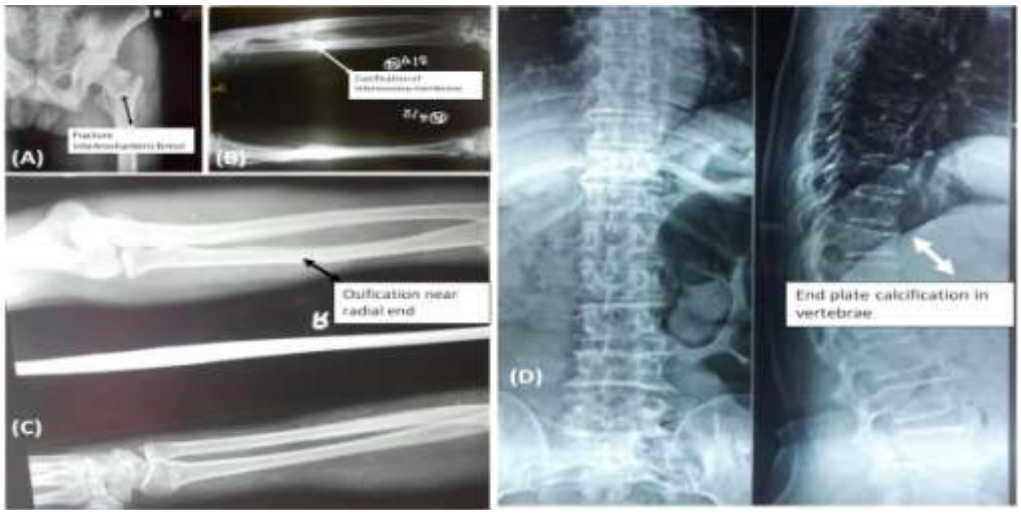

Fig. 2: Thickned trabeculae at femur with fracture intertrochanteric femur due to weak bone in young adult (A), Calcification at interosseous membrae in forearm; (B) Ossification of interosseous membrane at forearm bone with bowing; (C) and End plate ossification of lumber vertebrae with presence of coarse trabeculae (D).

DISCUSSION

Skeleton changes may result when drinking water content of fluoride exceeds 2 ppm.The estimated range of safe & adequate intake of fluorides in for adults is 1.5-4.0mg /day and it is less for children those with renal disease. In skeletal fluorosis patient does complaint vague discomfort, parethesias in limbs and trunk. There is stiffness at spine. The lumber spine is generally first to affect followed by thoracic & cervical spine. Restricted movement at spine is one of the earliest sign of skeletal fluorosis, although this may not be specific. The changes in skeleton caused by fluorosis can be evaluated by various methods. These methods includes, skeletal radiography, Bone densitometry (BMD), histomorphology, mineral maturity & crytallinity index, Quantitative bone histomorhometry, bone mass and Measuring bone fluoride content by bone biopsy (gold standard).

In skeletal radiography there are various changes which can be detected like osteosclerosis, osteopenia, osteoporosis. Osteosclerosis is evident when calcium intake is normal with continuous intake of fluoride for long term while osteoporotic form is more evident in paediatric age groups [4]. In osteosclerosis the bone is laid down in layers over each other, this excess deposition is due to stimulation of osteoblasts. But is this process fluoride substitutes the hydroxy group in hydroxyapatite crystals. These crystals are fine & binds with collagen in bone with increase avidity while the flouroapatite crystals are large in size thus decreases the total surface area, inturn it leads to decease in bone strength to stress [16, 17]. In our series we are able to see the fracture at intertrochanteric area which is most prone site for fracture due to bone weakness (Fig. 2).The osteoporosis may present as generalized osteopenia, osteomalacia is present as deformity in tubular bones like tiba, femur as genu varum, genu valgum or bow tibia. In our series most of the patient present with genu valgum deformity. These deformities are more liable to occur with fluoride level of 2-3 ppm [18]. Fluoride on skeletal appears to be mediated at several stage. It can directly interact with the bone mineral matrix biophysically [19]. Skeletal Fluorosis is a chronic metabolic disorder of bone caused by the exposure of fluoride in higher amount. Fluoride is a cumulative toxin that amplify metabolic turnover of the bone and impairs bone collagen synthesis [4]. Radiological studies of bone in fluorosis have shown irregular osteoid tissue deposited on the trabeculae and cortex, with extension into muscle attachments. It is reported here that the most radiologic features of this disease are increased bone mineral density, blur of trabeculae, thickening of bone and ossification of the attachments of tendons, ligaments, and muscles. The results of the present study are concomitant with the finding of Wang *et al*. [8]. Several studies have been proposed that that fluoride may increase bone mass results the newly formed bone appears to lack normal structure and strength [20]. It is suggested that high systemic fluoride exposures may lead to skeletal fluorosis, a condition hallmarked by osteosclerosis, ligament calcifications and osteomalacia which were confirmed by Wang *et al*. [21]. In addition, skeletal fluorosis can be intricate by malnutrition [22]. Increament bone density can be classified as osteopenia, borderline low density bone mass as well as osteoporosis, which are evidenced by low bone mass and microarchitectural deterioration of the bone tissue.

In the present study, a set of biochemical investigations were carried out. This revealed that increased level of calcium and phosphorus in serum of subjects than control. Calcium and Phosphorus are essential to human life. In vivo, the ionic forms of calcium and phosphorus combine to form calcium phosphate for bone. During the process of bone hardening, the Ca:P ratio gradually increases from 1:1 to 1.67. There are several stages of human life when the calcium and phosphorus requirements are most critical. It is believed that greatest peak bone mass laid down in the formative years leads to the greatest old age skeletal resilience and integrity. Also, fluoride has been reported to increase intestinal absorption of calcium and in osteoporosis, fluoride has been reported to increase intestinal absorption of calcium and phosphorus to improve calcium balance. Serum alkaline phosphatase levels were significantly raised in our series. The result was concomitant with the finding of Shashi and Bhardwaj [23]. Also, in vitro studies have shown that NaF elevate alkaline phosphatase activity [24].

Hydroxyproline is a major component of the protein collagen. Hydroxyproline and proline play key roles for collagen stability [25]. They permit the sharp twisting of the collagen helix. Vitamin D is essential to utilize dietary calcium efficiently. A serum 25(OH)D level of at least 20ng/ml is necessary to minimally satisfy the body's vitamin D requirement, and maintenance of a 25(OH)D serum level of 30 to 50ng/ml is recommended [26]. Adequate exposure to sunlight is the cheapest and most efficient method of preventing vitamin D deficiency. Another important result of our study was the finding of a relationship increase in symptomatic back pain and spinal BMD.

In conclusion, there is significant association between chronic exposure to fluorosis and presence of genu valgum deformity in lower limbs. In case of significant elevated Z AND T score fluorosis should be considered as one of the primary diagnosis. The alkaline phosphatase, hydroxyproline are elevated with

chronic exposure to fluoride but there elevation as a marker should only be established with presence of skeletal deformities. Increase in BMD and low vitamin D levels, which may contribute to an increased fracture risk, commonly occur in fluorosis.

CONCLUSION

On the basis of result it may be concluded that appropriate deflouridation measures with supplementation of vitamin D should be taken in affected areas with high occurrence of skeletal deformities with altered biochemical markers.

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