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Parathyroid Function in Osteofluorosis

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Abstract: The present study assessed the effect of fluoride on parathyroid function in 860 patients (mean age 32.50±10.50) affected with skeletal fluorosis, selected randomly from endemic fluorotic areas of district Bathinda, Punjab, India. The fluoride content in water sources was found to vary from 0.68-15.78 mg/L in study areas. Hence, the study areas were categorized as five different groups Control (0.68- 1.00 mg/L), A-I (1.01-4.00 mg/L), A-II (4.01-8.00 mg/L), A-III (8.01-12.00 mg/L) and A-IV (12.01-16.00 mg/L). An age and sex matched group of 140 control subjects without skeletal fluorosis were also included. The functional activity of the parathyroid was measured by radio immuno assay of parathyroid hormone (PTH). The biochemical estimations were made for serum and urinary fluoride, serum calcium, phosphorus, calcitonin and alkaline phosphatase (ALKP). The results revealed that level of serum and urinary fluoride was significantly ($p<0.001$) higher in fluorotic patients in comparison to control. The serum PTH, calcitonin and activity of ALKP was significantly ($P<0.001$) elevated in fluorotic patients. Significant ($P<0.05$) hypocalcaemia was observed in study group A-I and A-II and elevation in group A-IV. However, the alterations in calcium level in group A-III was statistically non significant. Hyperphosphatemia ($P<0.001$) was also observed in patients of fluorosis. Pearson's bivariate correlation showed positive correlation between water F vs serum F ($r= 0.98$, $P<0.001$), serum F vs PTH ($r= 0.97$, $P<0.007$), serum F vs calcitonin ($r=0.80$, $P<0.01$) and serum F vs ALKP ($r=0.93$, $P<0.02$). Negative correlation was noted between serum and urinary concentration of fluoride. When the serum fluoride concentration was increased the corresponding urinary fluoride excretion declined along with the advancing age. It may be concluded that high fluoride ingestion has a definite relation with increased calcitonin concentration, which may be the major cause of hypocalcemia in fluorotic patients, which may further leads to the increased parathyroid function i.e raised PTH levels in the serum to maintain serum calcium levels and may have a role in toxic manifestations of clinical and skeletal fluorosis.

Key words: ALKP · Fluorosis · Hypocalcemia · PTH

INTRODUCTION

Fluoride is a microelement for human health, it influences positively the dentition condition, abide on the other hand, the compound containing it have been listed among the most significant endotoxins that appear in natural environment. Fluoride ion, after absorption to the blood from alimentary tract, easily penetrates to the cells through membranes. Only a portion of it would be expelled from the body in urine. The most sustainable amount of fluorides is gathered in hard tissues. On the other hand, soft tissues are constantly saturated with them, as continuous flow of fluoride occurs [1]. Due to its strong electro negativity, fluoride is attracted by positively charged calcium ions in teeth and bones. Fluoride has been suggested to exert an effect on the calcium homeostatic system leading to secondary hyperparathyroidism and changes in parathyroid glandular structure and hormone secretion. Glandular hyperplasia has likewise been observed in humans living in areas of endemic fluorosis in India [2].

Fluoride is a cumulative toxin which can alter resorption of bone tissue. It also affects the homeostasis of bone mineral metabolism. A combination of osteosclerosis [3], osteomalacia and osteoporosis of varying degrees as well as exostosis formation

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characterizes the bone lesions. In a proportion of cases, secondary hyperparathyroidism has been reported with associated characteristic bone changes [4]. Increased metabolic turnover of the bone, impaired bone collagen synthesis and increased avidity for calcium are features in fluoride toxicity.

Parathyroid hormone (PTH) is an 84-amino acid polypeptide hormone and functioning as a mediator of bone remodeling and as an essential regulator of calcium homeostasis. PTH is secreted as a hormone in response to a hypocalcemic signal. PTH not only indirectly activates osteoclasts resulting in increased bone resorption but it has also been shown to be an anabolic factor mediating bone formation in skeletal tissue and *in vitro* [5, 6].

Calcitonin, plays an important role in regulating bone formation and affecting osteoblasts and believed to be involved in the mineralization of matrix due to the Gla-dependent binding of calcium and the subsequent absorption of hydroxiapetite. Therefore, calcitropic hormone might correlate with fluorosis and serum calcium [7]. The aim of this study to explore the status of parathyroid gland function to maintain calcium homeostasis and to investigate the relationship between various biochemical parameters involved in calcium homeostasis and fluoride concentration in water and body fluids in the people residing in fluoride endemic areas of Bathinda district, Punjab, India.

MATERIALS AND METHODS

Selection of Area: A detailed case control study was under taken to understand the etiology of the parathyroid function among the population in fluoride endemic areas. After analyzing the fluoride levels in drinking water, high fluoride areas have been selected for present study. The area with low fluoride level in drinking water was included in the study as a control. The selected areas were divided into five categories on the basis of water fluoride concentration viz: Control (0.76-1.00 mg/L), A-I (1.01-4.00 mg/L), A-II (4.01-8.00 mg/L), A-III (8.01-12.00 mg/L), A-IV (12.01-16.00 mg/L).

Subjects: The study group consisted of 860 subjects from different fluoride endemic areas and 140 control subjects from non fluorotic control areas were randomly selected. Skeletal grading was done by using the method of Teotia *et al*.[8]. Institutional Human Ethical Committee approved this epidemiologic investigation.

Sample Collection: 3-4 ml of fasting venous blood samples were collected in clean plastic centrifuge tubes. The blood was centrifuged for 15 minutes at 3000 rpm. Serum was separated and stored in deep freezer at -37°C until further analysis. Spot urine samples were collected in plastic bottles using toluene as a preservative from the selected patients and control.

Biochemical Analysis: The *in vitro* quantitative determination of parathyroid hormone and calcitonin in human serum samples (control and patients of fluorosis) was done on ELISA. Estimations were made for serum calcium, phosphorus and alkaline phosphatase with standard procedures using diagnostic kits.

Fluoride content of water, serum and urine samples was estimated with Orion ion selective electrode (EA940, Boston, MA, USA).

Statistical Analysis: All the data was expressed as Mean±SD. A comparison of various serum parameters was done between subjects of the fluoritic groups and the control group as well as among the subjects within the fluorotic groups. One way analysis of variance (ANOVA) with post-hoc analysis was used to compare the variables in different groups. Association between variables was assessed by Pearson's bivariate coefficient of correlation. Chi square analysis was used for non parametric data. Two sided P values of <0.05 were considered statistically significant. The statistical program used was SPSS for windows version 16.0 (Statistical Package for the Social Sciences inc., Chicago, Illinois, USA).

RESULTS

Mean fluoride content in drinking water sources of study group (Table 1 and Fig 1) and mean serum (SF) and urinary (UF) level of fluoride in patients were significantly (F_{SF}= 11.43,P<0.001; F_{UF}= 132.86, P<0.0001) higher than those of control group. Post hoc Tukey's LSD multiple comparison test revealed significant (q_{SF}= 0.30-0.56, 95% CI = -1.66 to 0.67; q_{UF} = -3.09 to -1.64, 95% = -10.37 to 4.95, P<0.05-0.0001) increase in fluoride level in body fluids. A positive correlation existed between drinking water fluoride and serum fluoride levels (r= 0.98, P<0.001, Fig. 3). However, the relationship between water F and Urinary F (r=0.24, P= 1.23) was statistically non-significant.

Clinical manifestations of different types of skeletal deformities were prevalent among different study groups (Fig. 2). The most of subjects-complaint pain and stiffness

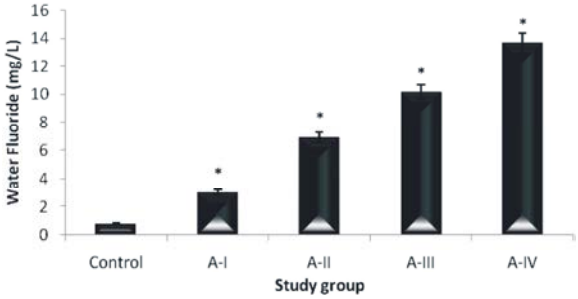

Fig. 1: Concentration of fluoride in drinking water of different study groups

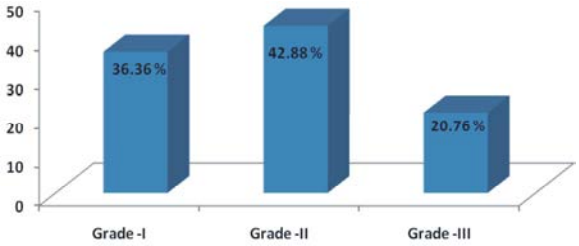

Fig. 2: Prevalence of different grades of skeletal fluorosis in fluorotic study groups

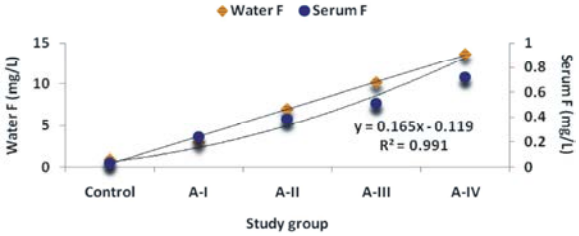

Fig. 3: Correlation between water F and Serum F in different study groups

in the joints, neck and exhibited signs of neck rigidity. In severe cases, the deformities prevented the subjects from assuming postures and performing movement of their choice, thereby crippling them. Chi-square analysis (X^2=63.760) revealed that maximum significant (P<0.001) numbers of cases were suffering from grade-II type of skeletal fluorosis. The grade-III (severe type) skeletal fluorosis was most prevalent in A-IV study group, where water fluoride content was highest (12.01-16.00mg/L). In control areas the situation differed in that there was absence of any kind of skeletal deformities.

Table 1: Concentration of fluoride in drinking water and body fluids in subjects of different study groups

Study group	Water F Range (mg/L)	Mean water F (mg/L)	Serum F (mg/L)	Urinary F (mg/L)
Control	0.76 - 1.00	0.84±0.19	0.02±0.004	0.89±0.23
A-I	1.01 -4.00	3.02±1.28*	0.32±0.028*	3.68±0.53*
A-II	4.01 -8.00	6.95±2.07*	0.37±0.019*	3.09±0.45*
A-III	8.01 - 12.00	10.18±1.83*	0.42±0.023*	2.86±0.41*
A-IV	12.01 - 16.00	13.67±2.04*	0.58±0.035*	2.23±0.32*

*P<0.001

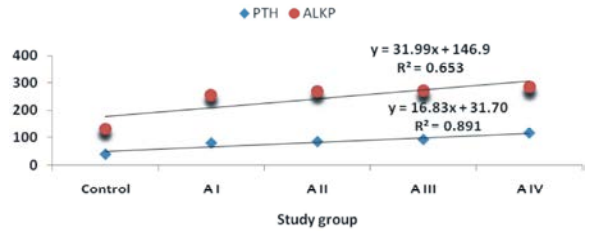

Fig. 4: Correlation between parathyroid hormone (PTH) and alkaline phosphatase (ALKP) in all study groups

One way ANOVA with post hoc Tukey's LSD multiple comparison test revealed that mean serum ALKP activity among the fluorotic subjects with skeletal deformities was significantly (F= 1923.66, q= 123.25 to 141.81; 95% CI = -476.80 to 246.51, P<0.05-0.0001) higher as compared to the control group (Table 2). The activity of ALKP was highest in patients of study group A-IV exposed to 12.01 – 16.00 mg/L of fluoride. ALKP showed positive correlation with water F (r =0.86, P<0.03) and serum F vs ALKP (r=0.93, P<0.02) and PTH vs ALKP (r= 0.86, P<0.01) Fig. 4.

Mean serum levels of PTH and calcitonin (CT) were significantly (F_{PTH} = 1513.42: P<0.0001; F_{CT}= 857.48: P<0.001) higher in patients of all fluoride study groups as compared to control. Post hoc Tukey's LSD multiple comparison test revealed significant (q_{PTH}= 40.76 to 77.66, 95% CI= -228.90 to 92.76; q_{CT}= 10.12 to 15.02, 95% CI= -49.68 to 20.81, P<0.05-0.0001) increase among the entire fluorotic study groups (Table 2). This also indicates that as the water fluoride concentration increases the level of PTH and calcitonin was also elevated. There was a positive correlation between serum fluoride vs PTH (r =0.97, P<0.007, Fig 5) and serum PTH vs calcitonin (r = 0.80, P<0.01, Fig 6) in different study groups.

Table 2: Biochemical parameters in control and fluorotic patients of different study group

Study group	ALKP (IU/L)	PTH (pg/ml)	Calcitonin (pg/ml)	Calcium (mg/dl)	Phosphorus (mg/dl)
Control	133.44±9.72	38.59±6.02	10.03±1.14	9.97±0.27	3.81±0.25
A-I	256.69±12.58*	79.35±8.35*	20.15±2.25*	8.66±0.29*	4.07±1.04
A-II	262.24±15.28*	84.47±10.31*	21.56±2.02*	8.79±0.20*	6.73±1.15*
A-III	269.54±18.35*	92.34±9.87*	24.55±3.01*	9.77±0.40	6.99±1.32*
A-IV	275.25±21.39*	116.25±11.25*	25.50±3.58	10.94±0.12	7.51±2.33*
F-value	1923.66	1513.428	857.428	2323.740	180.173

*P<0.001; F-value ANOVA

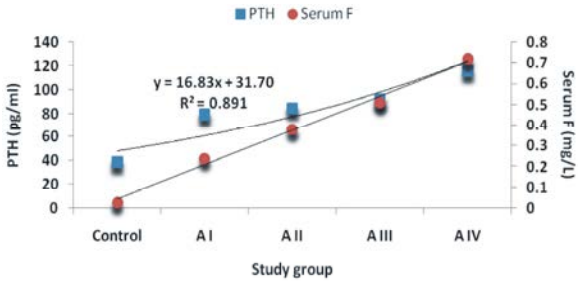

Fig. 5: Correlation between serum F and parathyroid hormone (PTH) in all study groups

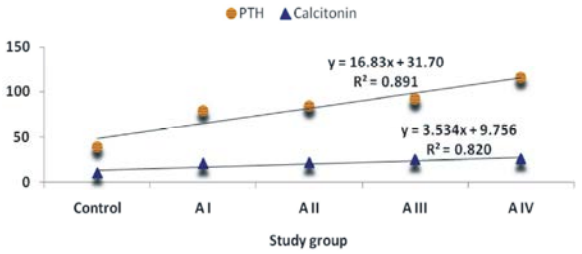

Fig. 6: Correlation between parathyroid hormone (PTH) and calcitonin in study groups

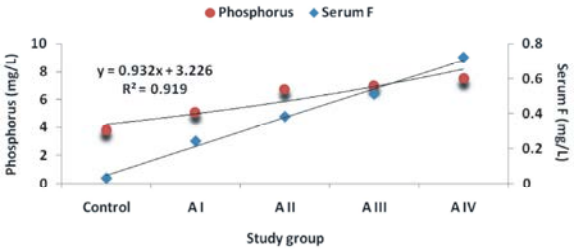

Fig. 7: Correlation between Serum F and phosphorus in study groups

One way ANOVA showed significant (F= 2323.74, P<0.01) alterations in serum calcium level (Table 2). Post hoc Tukey's multiple comparison test further illustrated that serum calcium levels in fluorotic subjects of A-I and A-II study group were significantly (P<0.05) lower than control group. In the study group A-IV, the calcium level was elevated (P<0.01) than that of control and other fluorotic groups. This may be due to maximum increase of PTH in these subjects. A positive correlation existed between serum F vs calcium levels in patients of all study groups, which was statistically non-significant (r=0.42, P= 0.89).

Hyperphosphatemia was observed in patients of all fluorotic study groups (Table 2). ANOVA with post hoc Tukey's LSD multiple comparison test showed significant (F= 180.173, q= 14.54 to 26.38, 95% CI= -11.38 to 7.30; P<0.05-0.0001) increase among all the fluorotic groups as well as compared to control, but in group A-I the difference was not statistically significant (P=0.87). Asignificant direct relationship existed between serum F vs phosphorus (r=0.95, P<0.01, Fig. 7)

DISCUSSION

The present study demonstrateed severe skeletal deformities in the people of high fluoride areas. The patients have complaint of neck rigidity and pain in knee and elbow joints. Earlier, we have reported that the deformities such as genu valgum and genu varum are associated with fluoride in drinking water [9].

In plasma, fluoride is transported as ionic fluoride and nonionic fluoride. Ionic fluoride does not bind to plasma proteins and is easily excreted with the urine. Kidney is the primary organ of excretion for fluorides. The amount of urinary fluoride excreted from the body reflects the amount of fluoride ingested. It has been documented that the urinary excretion of fluoride is 32–80% of total fluoride intake [10]. The biological half-life of bound fluoride is several years. The best indicator of fluoride exposure is the urinary fluoride concentration in the population under investigation. Different values for urinary fluoride levels (1.2–1.5 mg/L) were detected in skeletal fluorosis patients by Wang *et al* [11, 12]. In our study, mean urinary fluoride value was significantly (P<0.001) higher in patients with skeletal fluorosis, concordant with the literature.

The significant (P<0.001) elevation in serum levels of parathyroid hormone in fluoride exposed patients under the present study suggests alterations in parathyroid function.

The indirect action of fluoride on parathyroid hormone is relatively straight forward; fluoride induces a net increase in bone formation [13] and also decreases calcium absorption from the gastrointestinal tract beyond the degree expected by formation of calcium fluoride complexes, both of these effects lead to increase in the calcium requirement of the body [14]. If the dietary calcium is inadequate to support the increased requirement, the response is an increase in the synthesis of parathyroid hormone resulting in secondary hyperparathyroidism [4, 15].

Shashi *et al*. [16] suggested toxic effect of fluoride on the kidney as the cause of the hyperphosphatemia. The elevated PTH level resulting from calcium deprivation may be the primary signal, mediating the calcium regulation by increasing 1, $25(OH)_2D_3$ synthesis in the kidney [17]. Secondary hyperparathyroidism is thought to play an integral part in skeletal fluorosis. There are a

number of similarities between the effects of excess PTH and the administration of fluoride on bone. Gupta *et al*. [4] showed that high fluoride ingestion causes secondary hyperparathyroidism, which may be responsible for maintaining serum calcium levels and may play a role in causing toxic manifestations of fluorosis. Increased PTH correlated with urinary fluoride levels in children of endemic fluorosis area in Rajasthan. Some studies also indicate direct effects of fluoride on the parathyroid gland. Elevated parathyroid hormone in the presence of normal serum calcium might indicate a stimulatory effect of fluoride [4, 15]. The absence of the normal elevation of parathyroid hormone in response to calcium deficiency suggests an inhibitory effect [18].

In the present study it was also observed that the level of calcitonin was significantly ($P < 0.001$) increased with increase in fluoride concentration in drinking water. The rise in serum calcitonin and fluoride may affect kidney function in such a way as to result in a slight decrease in glomerular filtration rate (GFR). This may partially explain the rise observed in phosphorous; a reduction in GFR increases the phosphorous threshold concentration, resulting in increased plasma phosphorous [19]. Calcitonin on the other hand is known to cause phosphaturia without significantly affecting GFR in normal kidneys [20]. Xu et al. [21] evaluated the level of serum PTH when rats were exposed to excessive fluoride with low-calcium diet. Cotreatment of excessive fluoride with low-calcium diet largely stimulated the secretion of PTH. The levels of serum PTH increased gradually with the extension of fluoride exposure; on the contrary, the concentration of serum calcium decreased gradually, both of which embodied a time-dependent relationship and demonstrated that fluoride by itself affected the body's calcium metabolism and stimulated the secretion of PTH. They inferred that fluoride partly acted by changing the levels of circulating calcium on regulating the secretion of PTH as reported in the present study.

In vitro and *in vivo* studies showed that calcitonin was effective in inhibiting osteoclast activity, thereby reducing bone resorption [22]. Wallach *et al*. [23] reported in an animal model that calcitonin increased bone growth and biochemical markers of bone formation. In the present study, the serum calcitonin levels among the fluorotic patients were higher than that of control group. It suggested that the increased serum concentration of calcitonin reducing bone resorption caused by high fluoride. Rao [24] believed that calcitonin would be decreased and the relationship between thyroxine and some other calcium related hormones such as calcitonin would be imbalance when blood thyroxine decreased. Iodine may improve the calcitonin concentration by regulating the concentration of blood thyroxine. On the other hand, the level of calcitonin is closely related to the level of calcium. The higher levels of calcium, the higher level of calcitonin [25].

Our results showed a highly significant ($P<0.001$) increase in alkaline phosphatase activity in fluorotic patients of all age groups as compared to control.

The serum level of alkaline phosphatase is an index of bone activity. High levels of alkaline phosphatase may suggest the presence of bone disturbances resulting in bone destruction and are associated with the osteoblastic activity of the bone. High values of alkaline phosphastase activity have been reported to be associated with fluorosis, which increased osteoblastic activity [13, 26, 27]. In another study conducted in an fluorotic endemic area in India, Misra *et al*. [28] demonstrated the mean levels of serum alkaline phosphatase to 229.70±123.05 IU/L in adults (the corresponding level in the present study was 263.70±19.67 IU/L). Harinarayan *et al*. [18] reported hypocalcemia and higher levels of serum alkaline phosphatase (264.5±222.5 IU/L) in fluorotoxic metabolic bone disease patients due to excess fluoride ingestion. Khandare *et al*. [13] estimated higher activity of alkaline phosphatase in children exposed to high fluoride in drinking water.

Ravichandran *et al.* [29] observed higher alkaline phosphatase activity (236.37±138.68 IU/L) in 259 subjects residing in a fluoride effected area in India. The alterations in bone metabolism in fluorosis seem to be multifactorial. Deposition and resorption of bone increase, presumably because of over production of parathyroid hormone. Elevated plasma alkaline phosphatase and parathyroid hormone levels support the diagnosis of associated metabolic bone disease and secondary hyperparathyroidism in fluorosis [4, 13, 15,]. However, normal levels of alkaline phosphatsase activity has also been reported [30].

In the present study, a inverse correlation occurred between serum parathyroid hormone levels and serum calcium. Studies have documented that ingestion of fluoride causes a decrease in the serum ionic calcium [28, 30]. The resultant hypocalcemia leads to secondary hyperparathyroidism [31]. Any cause of hypocalcemia or vitamin D deficiency can lead to secondary hyperparathyroidism in an attempt by the body to maintain calcium homeostasis [15, 32]. Secondary

hyperparathyroidism in response to calcium deficiency may contribute to a number of diseases, including osteoporosis, hypertension, arterosclerosis, degenerative neurological disease, diabetes mellitus some form of muscular dystrophy and colorectal carcinoma [33].

In conclusion, this study provides evidence that hyperfunctioning of parathyroid gland may modify the serum calcium levels related to fluorine exposure. This suggests that a new biomarker of dental fluorosis may be used to identify high-risk populations in areas with high levels of fluoride in drinking water. The study shows the increased PTH levels because of endemic waterborne fluorosis, with the existence of its most common reason; hypocalcemia and hyperphosphatemia along with raised level of calcitonin are interrelated. It may be concluded that high fluoride ingestion has a definite relation with increased calcitonin concentration, which may be the major cause of hypocalcemia in fluorotic patients, which may further leads to the increased parathyroid function i.e raised PTH levels in the serum to maintain serum calcium levels and may have a role in toxic manifestations of clinical and skeletal fluorosis.

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