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CHANGES IN SERUM SEROMUCOID FOLLOWING COMPENSATORY HYPERPARATHYROIDISM: A SEQUEL TO CHRONIC FLUORIDE INGESTION

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ABSTRACT

This study was conducted to find out the possible underlying mechanism of various manifestation of fluorosis, a disease caused by excess ingestion of fluoride. For this the fluoride belt of Jaipur district was selected. The parameters selected were serum Parathyroid hormone, the levels of which are directly affected by fluoride intake. The levels of serum seromucoid, serum and leucocyte ascorbic acid, serum sialic acid (SSA) reflects ground substance metabolism. The study was conducted on two hundred children, selected from four areas (50 from each area) consuming water containing 2.4, 4.6, 5.6 and 13.6 mg/l of fluoride. Drinking water fluoride and serum fluoride were measured by Ion selective electrode method. Serum parathyroid by RIA and all other parameters were measured spectrophotometrically. The results revealed an increase in levels of fluoride, parathyroid hormone and seromucoid in serum with increasing water fluoride concentrations. Serum Calcium and serum ascorbic acid were found in normal range, how ever leucocyte ascorbic acid were decreased. A high positive correlation among fluoride concentration in drinking water and serum parathyroid hormone (r=0.967), and, serum parathyroid hormone and serum seromucoid concentration (r=0.935) was also observed The results indicated that secondary hyperparathyroidism due to hypocalcemic stress caused by excess fluoride ingestion disturbs normal metabolism of ground substance in calcified tissues of the body reflected as altered levels of the components of ground substance in the serum.

KEY WORDS

Fluorosis, Sialic acid, Glycosaminoglycans, Seromucoid, Drinking water

INTRODUCTION

Systemic fluorosis is an endemic problem in several developing countries including India and Pakistan and has also been reported sporadically in other parts of the world (1). While the WHO guidelines permit only 1.5 mg/l (ppm) as a safe limit for human consumption (2), people in seventeen states of India are consuming water with fluoride concentrations even up to 44 mg/l (3). Toxic effects of excessive fluoride ingestion (1, 4, 5) manifests in three forms: clinical, skeletal and dental. General manifestations are: dental discoloration, dental as well as skeletal deformities, severe joint pains, general debility, as also psychosocial problems due to bad teeth, body deformities and immobility.

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Studies conducted on human and animals to evaluate the effect of fluoride ingestion on mucopolysaccharide and parathyroid hormones (PTH) reported a change in sulphation pattern of glycosaminoglycans in many tissues of the body including bones.(6-8), which in turn greatly enhances the leaching of calcium from bones (bone resorption) and also increases the levels of hyaluronic acid in bone and other tissues of the body (9-11). The results of these studies were, however inconclusive. Therefore, the present study was planned to evaluate the effect of chronic fluoride ingestion on serum parathyroid hormone and mucopolysaccharide (seromucoid) and any possible correlation between these parameters.

MATERIALS AND METHODS

Drinking water fluoride concentration was determined in 50

villages of Jaipur (India). Out of these, four areas were selected based on different fluoride concentrations. In remaining 46 villages fluoride concentration was less then 1.5 ppm hence they were excluded from the study.

Group A	Ramsagar ki Dhani	2.4 ppm
Group B	Rampura	4.6 ppm
Group C	Shivdaspura	5.6 ppm
Group D	Raipuria	13.6 ppm

The criteria for the selection was based on levels of fluoride content above 1.5 ppm in drinking water in these villages, proximity to the investigators working place and compliance of the children to therapeutic intervention.

From each of these areas fifty male children manifesting dental fluorosis were selected randomly for inclusion in study. All the children were ranging in age from 6 to 12 years (school going), their body weight ranging from 18 to 30 kg. These children were graded for clinical (non skeletal), skeletal (radiological) and dental fluorosis (12,13). An informed consent was obtained from parents of all children after explaining the purpose of the study. Requisite amount of blood was drawn in the morning for the tests. Concentration of fluoride in drinking water and serum was measured using ion selective electrode method using Orion's pH/ISE meter, model 920A (14). The measurements were done as detailed in the booklet. Mid molecule parathyroid hormone was measured by radio immuno assay (15) using kit supplied by Incstar (Incstar Corporation – Stillwater, Minnesota, USA). Serum seromucoid (16), Serum Sialic Acid (17), Serum ascorbic acid (18) and Leucocyte ascorbic acid(19) were measured spectro photometrically.

Statistical Analysis : Coefficient of correlation(r) was applied using Microsoft Excel software, Microsoft Corporation, USA on a personal computer. The p-value for significance of correlation analysis were also calculated (p value < 0.05 was considered significant). The relevant p values of correlation analysis (r) is discussed.

RESULTS

The observations related to grading of dental and skeletal fluorosis are depicted in Table 1. The severity (represented in terms of average) of dental fluorosis (on Dean's scale) and skeletal fluorosis observed in these areas was: Ram sagar ki dhani; 2.71 & 0.68, Rampura; 1.73 & 0.5, Shivdaspura ; 2.44 & 0.79 and Raipuria ; 3.43 & 0.9.

Mean and Standard deviation of parathyroid hormone, fluoride, sialic acid, ascorbic acid and seromucoid in serum and leucocyte ascorbic acid are also given in Table 1, which shows that fluoride, parathyroid and seromucoid concentrations in serum increased with increasing water fluoride contents but at the same time serum and leucocyte Ascorbic acid decreased.

DISCUSSION

Fluoride concentrations in serum were high in all the areas included in this study as compared to the reported normal values (Table 1). Other workers (20) also observed similar findings. The severity of skeletal and dental fluorosis in study areas ranged from 0.68 to 0.9 and 1.73 to 3.43 (represented as an average for the area) respectively. The severity is increasing with increasing fluoride concentration in drinking water with the exception of area B, where the severity of skeletal and dental fluorosis (0.5 and 1.73) is lesser than that of area A despite higher drinking water fluoride concentration. This could be due to higher serum and leukocyte ascorbic

Table 1 : Water fluoride, Serum fluoride, Serum Calcium, Serum Parathyroid, Serum Seromucoid (SSM), Serum Sialic Acid, Ascorbic Acid (Serum and Leucocute) and Fluorosis grading in Different areas with different fluoride concentrations (Values are Mean±SD)

Name of village	Water fluoride	Serum Fluoride	Serum Calcium	S.PTH-MM II	SSM	SSA	Ascorbic acid		Dental Fluorosis	Skeletal Fluorosis
							Serum	Leukocyte		
	mg/L	mg/L	mg/dl	pmol/l	mg/dl	mg/dl	mg/dl	mg/10^8 WBC	Dean's Scale	in term of Grades
Normal levels	< 1.5	0.13-0.17	8.8 – 10.8	37 – 56	9-11	59-64	0.4 – 1.4	24 – 40		
Ram sagar Ki Dhani (n = 50)	2.6	0.79±0.21	9.23±1.89	31.64±2.82	16.94±4.88	28.31±2.06	1.09±0.82	24.83±9.37	2.71±1.09	0.68±0.67
Rampura (n = 50)	4.6	1.10 ±0.58	10.75±1.66	40.98±26.9	18.59±4.56	66.59±3.25	1.29±1.03	28.93±15.29	1.73±1.09	0.5±0.61
Shivdaspura (n = 50)	5.4	1.10 ±0.17	9.68±0.99	75.07±31.75	19.7±9.63	24.97±1.13	0.90±0.58	15.60±8.44	2.44±1.32	0.79±0.91
Riapuria (n = 50)	13.6	1.07±0.17	10.39±1.44	125.10±131.14	39.88±7.04	25.18±1.86	0.66±0.40	9.50±6.66	3.43±1.70	0.95±1.12

acid levels in area B (Table 1) providing a protective effect (21).

The levels of serum parathyroid hormone were well within normal range (48.1± 11.9 pmol/L), in group A and B whereas in groups C and D the levels were higher, probably due to relatively higher quantity of ingested fluoride resulting in increased hypocalcemic stress (22). Increase in Serum parathyroid hormone levels with increasing fluoride ingestion has also been reported by other workers (7,8). The serum calcium is within the normal range in all groups, maintained due to secondary hyperparathyroidism. A statistical analysis indicated high positive correlation (r= 0.967) between serum parathyroid hormone and fluoride concentration in drinking water.

Elevated levels of serum seromucoid (SSM) have been observed in all studied areas. The maximum elevations have been observed in Group D, consuming highest fluoride concentration in drinking water. Serum seromucoid concentration showed a significant positive correlation with serum parathyroid hormone (r= +0.935).

Lower levels of Sialic acid is observed in all areas, except in group B. This could be due to higher serum and leukocyte ascorbic acid levels in area B providing a protective effect. Measurement of Sialic acid is an important parameter in detection of fluoride toxicity. Its levels are decreased in serum as a result of F-toxicity both in animal and man (23).

Although the levels of serum ascorbic acid (SAA) are within normal range, but lower levels have been observed in children of areas having higher fluoride content in drinking water. The leukocyte ascorbic acid levels were below the normal range in all areas except in area B. The low levels of LAA indicate the longstanding depletion in ascorbic acid, in comparison to SAA. These observations are consistent with the observations of other workers (24,25,26).

The ground substance of bone is regarded as a highly polymerized negatively charged macromolecules glycoprotein (22) Which is relatively insoluble and chemically inert, under some conditions, but becomes highly labile under certain physiologic and pathologic states (27,28,29). Biochemical parameters included in this study are forming a constituent part of the ground substance of the calcified tissues of the body or are involved in its metabolic turnover. Many studies have reported increased levels of parathyroid hormone in population consuming high concentration of fluoride in drinking water. Parathyroid hormone has been shown to influence the metabolism of ground substance in calcified tissues of the body. (7,8) In present study levels of serum seromucoid and sialic acid are altered as compared to normal levels, more in areas where the levels of parathyroid hormone are higher indicating that raised parathyroid hormone disturbs the ground substance composition by causing depolymerization of the proteoglycane macromolecule with concomitant decreased calcium binding leading to decalcification. The studies indicates that alteration of the glycoprotein constituents of bone and of epiphysial cartilage follows the administration of parathyroid extract (30). It appears that the inorganic elements are released concurrently with the depolymerization of the ground substance. The diffusible depolymerized fragments enter the blood, where they are detected as an elevation of the serum seromucoid levels. The decrease in the levels of sialic acid in serum has also been reported by some workers (23) while others found no change (17). In present study also there is a significant positive correlation between serum parathyroid hormone levels and serum seromucoid. There are reports indicating involvement of fluoride and parathyroid hormone in bone metaboligm (28, 29, 31). Further, injestion of fluoride causes a decrease in the serum ionic calcium (32). The resultant hypocalcemia leads to secondary hyperparathyroidism (33), which is responsible for various manifestations of fluorosis like, delayed eruption of teeth, dental, skeletal and clinical fluorosis as well as premature aging etc. (34).

The study, indicates positive correlation between fluoride concentration in drinking water and serum parathyroid hormone and seromucoid levels.

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REFERENCES

1. Susheela A K. “Prevention and Control of Fluorosis”, Technical Information for Training cum Awareness Camp for Doctors, Public Health Engineers and other Officers, Published by National Technology Mission of Drinking Water, New Delhi, 1991.

2. WHO. Guidelines for Drinking Water quality, Vol. 2, World Health Organisation, Geneva 1984: 249pp.
3. PHED Survey. Fluoride Affected Villages /Habitation.1991-93.
4. WHO. "Fluorides and Human Health", Monograph Series No. 59, 1970.
5. WHO. Fluorine and Fluoride, (Environmental Health Criteria 36). World Health Organization, Geneva, 1984: 93pp.
6. Bronner F. Parathyroid effects on sulfate metabolism: interrelationship with calcium. In: Greep RO, Talmage RV and Charles C Thomas, ediors. The parathyroids. Springfield, Illinois, USA, 1961:123-43.
7. Bordier PHJ, Chot ST. Quantitative histology of metabolic bone disease. In: Mac Intyre I, editor. Clinics in Endocrinology and Metabolism. WB Saunders company ltd. London. 1972, 1(1): 204-13.
8. Cramer CF, Suiker AP, Copp DH. Effect of Parathyroid extract on glycoprotein and polysaccharide component of serum and tissue. In: Greep RO, Talmage RV and Charles C Thomas, ediors. The parathyroids. Springfield, Illinois, USA, 1961:144-57.
9. Mayes PA. Carbohydrates of physiologic significance. In: Murray RK, Granner DK, Mayes PA and Rodwell VW, editors. Harper's Biochemistry, 25th edn,. Appleton & Lange. Stamford, Connecticut, 2000: 149-59.
10. Canon DC, Olitzky I, Inkpen JA. Proteins. In: Henry RJ, Canon DC and Winkelman JW, editors. Clinical Chemistry – Principals and Technics. 2nd edn, Harper and Row publishers, Newyork, 1974: 405-502.
11. Murray RK, Keeley FW. The extracellular matrix. In: Murray RK, Granner DK, Mayes PA and Rodwell VW, editors. Harper's Biochemistry, 25th edn, Appleton & Lange. Stamford, Connecticut. 2000: 695-714.
12. Teotia SPS, Teotia M, Singh DP, editors. Bone static and dynamic histomorphometry in endemic fluorosis. In: Fluoride Research 1985, studies in Environmental Science, vol. 27. Elsevier science publishers BV, Amsterdam, 1985: 347-55.
13. Dean HT. Classification of mottled enamel diagnosis. J Am Dent Assoc 1934: 21:1421-26.
14. Fuchs C, Dom D, Fuchs CA, Henning HV, Mecintosh C, Scheler F. Fluoride determination in plasma by ion selective electrode: a simplified method for the clinical laboratory. Clin Chim Acta 1975:60: 157-67.
15. Lindall AW, Ells JE, Roos B. Estimation of biologically active intact parathyroid hormone in normal and hyperparathyroid sera by sequential N-terminal immunoextraction and midregion radioimmunoassay. J Clin Endocrinol Metabol 1983; 57: 1007.
16. Weimer HE, Mohsin JR. Seromucoid estimation using orcinol reaction adopted by Rimington. Am Rev Tuberc Pulmonary Diseases 1952; 68: 594.
17. Seibert FB, Pfaff ML, Seibert MV. Serum sialic acid estimation by tryptophane perchloric acid reaction. Arch Biochem1948; 18: 279.
18. Varley H, editor. Determination of ascorbic acid in blood and plasma. Practical clinical biochemistry, 4th edn, William Heinemann Medical books Ltd. and Interscience books Inc. New York, 1975: 635-637pp.
19. Denson KW, Bowers EF. The determination of ascorbic acid in white blood cells. Clin Sci 1961; 21: 157-58.
20. Shusheela AK, Jha M. Effect of fluoride on glycosaminoglycans of cancellous and cortical bone of rabbit. Experientia 1981; 37: 1097-99.
21. Gupta SK, Gupta RC, Seth AK, Gupta A. Reversal of fluorosis in children, Acta Paediatrica Japonica 1996; 38: 513-19.
22. Khandare AL, Harikumar R, Sivakumar B. Severe bone deformities in young children from vitamin D deficiency and fluorosis in Bihar-India. Calcif Tissue Int 2005 Jun;76(6): 412-8
23. Jha M, Shusheela AK, Neelam Krishna, Rajyalaxmi K, Venkiah K. Excessive ingestion of fluoride and the significance of sialic acid: glucosaminoglycans in the serum of rabbit and human subjects. Clin toxicol 1983; 19(10): 1023-30.
24. Rao RL. Recent advances in research on fluoride toxicity and fluorosis. ICMR bulletin vol. 3(March) , Indian Council of Medical Research 1979: 1-4pp.
25. Pandit CG, Raghavachari TNS, Rao DS, Krishnamurti V. Endemic fluorosis in South India: A study of the factors involved in the production of mottled enamel and severe bone manifestations in adults. Indian Journal of Medical Research 1940;28:533-58.
26. Jenkins GN, Venkateswarlu P, Zipkin I. Physiological effects of small doses of fluoride. In: Fluoride and human health, Geneva , World Health Organization. 1970: 177-9 pp.
27. Cobb J . The morphological distribution of Glycogen and Glycoproteins in the cells and Extra Cellular Materials of Growing Bones, M.S. Thesis, University of Illinois, 1948.
28. Armstrong WD, Messer H, Singer L. Effect of bone fluoride on bone resorption and metabolism. In: Friedrich Kuhlen cordt and Hans Peter Kruse, editors. Calcium Metabolism, bone and metabolic bone diseases. Proceedings of the X European Symposium on calcified tissues, Hamburg (Germany), 16-21 September 1973, Springer-verlag Berlin. Heidelberg. Newyork, 1975: 132-133pp.

29. Jowsey I, Riggs BL, Kelly PJ . Long term experience with fluoride and fluoride combination treatment of osteoporosis. In: Friedrich Kuhlen cordt and Hans Peter Kruse, editors. Calcium Metabolism, bone and metabolic bone diseases. Proceedings of the X European Symposium on calcified tissues, Hamburg (Germany), 16-21 September 1973, Springer-verlag Berlin. Heidelberg. Newyork, 1975: 151-154pp.

30. Engel MB. Mobilization of mucoprotein by parathyroid extract. A.M.A Archieves of Pathology. 1952; 53: 339-51.

31. Harinarayan CV, Kochupillai N, Madhu SV, Gupta N, Meunier PJ. Fluorotoxic metabolic bone disease: an osteo-renal syndrome caused by excess fluoride ingestion in the tropics. Bone 2006 Oct;39(4):907-14.

32. Srivastava RN, Gill DS, Moudgil A, Menon RK, Thomas M, Dandona P. Normal ionised Calcium, Parathyroid hypersecretion, and elevated Osteocalcin in a family with Fluorosis. Metabolism 1989; 38 (2): 120-24.

33. Mikhailova NN, Anokhina AS, Ulanova EV, Fomenko DV, Kizichenko NV. Experimental studies of pathogenesis of chronic fluoride intoxication. Patol Fiziol Eksp Ter 2006; (3):19-21.

34. Waddington RJ, Embery G, Hall RC. The influence of fluoride on proteoglycan structure using a rat odontoblast in vitro system. Calcif-Tissue-Int 1993 (May); 52(5): 392-98.