INVESTIGATIONS OF SOFT TISSUE FUNCTIONS IN FLUOROTIC INDIVIDUALS OF NORTH GUJARAT

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SUMMARY: The present study was undertaken to investigate the various health problems caused by water-borne fluoride in endemic villages of Mehsana and Banaskantha districts of Gujarat. The study revealed high levels of fluoride in serum samples of the villagers. Mottling of teeth and skeletal complications were common. Intake of fluoride caused a decrease in hæmoglobin content and in the serum protein levels. Serum cholesterol levels were normal. Circulating levels of testosterone were decreased, but not significantly enough to indicate an effect on reproductive functions. The enhanced levels of serum transaminases, which are markers for liver function, indicated structural and functional changes in liver due to fluoride intake. Changes in the serum calcium, sodium and potassium levels revealed electrolyte imbalance in the fluorotic individuals. While levels of thyroid stimulating hormone (TSH) and triiodothyronine (T_3) did not vary, a significant increase in the thyroxine (T_4) levels suggested alteration in thyroid function. Thus the study revealed some harmful effects of fluoride in the soft tissue functions of the endemic population.

Key Words: Banaskantha; Mehsana; Soft tissue function; Water-borne fluoride.

Introduction

Fluorosis caused by intake of fluoride has been recognised in India for six decades. It is a slow, progressive crippling malady, affecting young and old, rural and urban populations. It has recently attained alarming dimensions. It is widespread in as many as 15 states of the Indian Republic, afflicting some 25 million people. Gujarat is one of the 15 states in India reported to have endemic fluorosis, and 18 out of the 19 districts have elevated fluoride concentrations in drinking water. The worst affected districts include Amreli, Mehsana, Banaskantha, Sabarkantha and Baroda (Chhota Udepur).

Agricultural crops, water and food are contaminated with fluoride because of the high concentrations of fluoride in the soil of those regions.

The Geological Survey of India reveals that topaz, apatite, rock phosphate, phosphate nodules and phosphorite are widespread in the earth's crust in India and contain high percentages of fluoride. As a result of the rich mineral content and high rainfall, fluoride leaches out and contaminates the water and the soil.

Fluoride is known to affect the dental and skeletal systems. In addition, studies carried out in the past few years have shown detrimental effects on soft tissue functions of various animal models.[1-7] Its effect on soft tissue functions on humans is less understood and few reports are available regarding this aspect of fluoride toxicity. We have carried out surveys in the fluoride endemic areas of Gujarat since

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1985. This paper reports our surveys during the last three years. Earlier survey results have been published.[8,9]

Materials and Methods

Five hundred individuals from fifty-two villages in Mehsana and Banaskantha of Gujarat State were initially examined. Individual proforma sheets recorded the following: Name, age, sex, address, drinking water source, depth of the well or pond, duration of stay in the village, food habits, dental changes and skeletal changes. The tests of Susheela [10] were used to assess whether or not the joints were affected.

After collection of those data, drinking water source samples, and blood collected from randomly selected fluorotic individuals, were brought to the laboratory for analysis. The serum was separated by centrifugation.

Selection of control individuals:

Water samples and blood samples were also collected from Ahmedabad city where water fluoride is below the permissible limit (Indian Bureau of Standards).

The following parameters were studied in control and fluorotic populations:

Fluoride in drinking water and serum samples were determined with an Ion selective electrode Orion. Model 701A, and were expressed as ppm.

Hæmoglobin levels, determined with a hæmometer, were expressed as gm%.

Serum protein levels were estimated by the method of Gornall *et al*,[11] and expressed as gm/100 mL serum.

Serum cholesterol levels, determined by the method of Pearson *et al*,[12] were expressed as mg/100mL.

Serum glutamate pyruvate transaminase (SGPT) levels were estimated by the method of Reitman and Frankel[13] and expressed as mU/mL serum.

Serum glutamate oxaloacetate transaminase (SGOT) levels were estimated by the method of Reitman and Frankel[13] and expressed as mU/mL serum.

Calcium, sodium and potassium levels in serum were estimated by a Flame Photometer (Systronic digital unit type 125) according to the method of Dean [14] and expressed as ppm.

Serum hormones:

Testosterone serum levels were assayed by the double antibody radioimmunoassay (RIA) technique of Peterson and Swerdloff[15] (kit obtained from Binax, USA) and were expressed as ng/mL serum.

Triiodothyronine and thyroxine (T_3 and T_4) serum levels were determined by RIA method of Peterson and Swerdloff[15] (kits obtained from BARC Bombay) and were expressed as ng/mL.

Thyroid stimulating hormone (TSH) serum levels were determined by the immunoradiometric assay method of Kemp *et al*[16] (kit supplied by BARC, Bombay) and were expressed as μU/mL serum.

Serum catecholamines levels were determined by the method of von Euler and Hamberg[17] and expressed as μg/mL serum.

Results

Surveys conducted in 52 villages of North Gujarat revealed symptoms of fluorosis in the majority of the individuals studied. 74% of the individuals showed slight to severe mottling of teeth. 59% had stiffness of spinal cord. Other skeletal problems such as stiff hands and fingers (60%), stiffness of legs and joints (65%) were also common. 93% of the cases studied were regular tea drinkers. Tea accumulates fluoride, so may contribute to the fluoride burden of the body.

Water fluoride content: Water samples from various places of Ahmedabad city showed fluoride levels within the permissible limit (mean: 0.6 ppm). However, as before,[8,9] the samples collected from endemic villages of North Gujarat revealed high fluoride contents, ranging in this survey (Table 1) from 1.0 to 6.53 ppm. Of the 52 villages 18 (35%) had fluoride content below 2 ppm and 26 (50%) had fluoride content within the range of 2-4 ppm while 8 (15%) had fluoride levels above 4 ppm. Bore water samples had higher fluoride concentrations than well water samples.

Tables 1-4 present similar results to those of earlier surveys,[8,9] *i.e.* the fluorotic individuals, when compared to controls, had significantly increased serum levels ($P < 0.001$) of fluoride, SGOT, SGPT, sodium and potassium, and serum cholesterol levels were essentially the same in both populations.

In this survey, there was an insignificant decrease in mean serum testosterone levels in the fluorotic individuals, compared to controls.

Other new results from this survey were:

Serum fluoride: Of the 80 samples analysed, 38% had fluoride concentration < 0.2 ppm; 47% showed F^- levels in the range 0.2-0.4 ppm; while 15% showed F^- levels above 0.4 ppm (Table 1). Fluoride concentration increased with age (see Figure).

Hæmoglobin: Levels of the fluorotic group showed an insignificant decrease when compared to the control population (Table 2).

Serum cholesterol: Levels were essentially the same for both fluorotic populations and controls (Table 2).

Serum protein and calcium: Levels of the endemic population showed a highly significant decrease ($p < 0.001$) as compared to the control (Tables 3 and 4).

Serum triiodothyronine (T3) and *thyroid stimulating hormone* (T5): There was no difference in levels in the fluorotic and control groups (Table 5).

Serum thyroxine (T4): Levels showed a significant increase ($P < 0.001$) compared to control (Table 5).

Serum catecholamines: The serum adrenalin and nor-adrenalin levels increased significantly in fluorotic individuals ($P < 0.001$) compared to controls (Table 6).

TABLE 1. Water and serum fluoride levels (ppm) in control and endemic population

Parameter	Control	Endemic Population
Water fluoride	0.638 ± 0.013	2.70 ± 0.18
Range	0.56 - 0.72	1.0 - 6.53
No. of cases	15	52
Serum fluoride	0.04 ± 0.002	0.284 ± 0.032
Range	0.03 ± 0.05	0.131 ± 0.552
No. of cases	15	76

Values are Mean ± S.E.

TABLE 2. Hæmoglobin (gm%), serum cholesterol (mg/100 mL) and testosterone (ng/mL)

Parameter	Control	Endemic Population
Hæmoglobin	13.3 ± 0.339	12.89 ± 1.48
Range	11.8 - 16.4	7.8 - 16.0
No. of cases	22	75
Cholesterol	155.22 ± 7.51	148.25 ± 3.90
Range	122.5 - 200	64.0 - 192.30
No. of cases	15	40
Testosterone	6.42 ± 0.423	5.56 ± 0.49
Range	4.3 - 9.3	1.10 - 11.5
No. of cases	15	35

Values are Mean ± S.E.

TABLE 3. SGOT, SGPT (mU/mL) and serum protein (gm/100 mL) levels

Parameter	Control	Endemic Population
SGOT	16.02 ± 1.2	29.38 ± 0.83
Range	12.0 - 24.0	23 - 41
No. of cases	15	35
SGPT	11.7 ± 0.83	22.33 ± 0.73
Range	9 - 15	15 - 29
No. of cases	15	35
Protein	8.64 ± 0.144	5.76 ± 0.89
Range	7.942 - 9.42	4.15 ± 6.98
No. of cases	15	35

Values are Mean ± S.E.

TABLE 4 - Calcium, sodium and potassium levels (in ppm)

Parameter	Control	Endemic Population
Calcium	0.958 ± 0.045	0.595 ± 0.014
Range	0.71 - 1.15	0.40 - 0.79
No. of cases	15	60
Sodium	1163.68 ± 28.84	1875.59 ± 30.8
Range	861 - 1500	1320 - 2450
No. of cases	22	60
Potassium	129.35 ± 7.96	322.75 ± 42.38
Range	90 - 120	130-820
No. of cases	22	60

Values are Mean ± S.E.

TABLE 5. T_3, T_4 and TSH levels in serum

Parameter	Control	Endemic Population
T3 (ng/mL)	1.50 ± 0.135	1.528 ± 0.076
Range	0.70 - 2.1	1.0 - 3.7
No. of cases	15	40
T4 (ng/mL)	9.16 ± 0.63	14.77 ± 0.512
Range	5.4 - 13.0	7.2 - 20.0
No. of cases	15	40
TSH (μU/mL)	2.56 ± 0.36	2.55 ± 0.37
Range	0.50 - 4.4	0.30 - 6.1
No. of cases	15	55

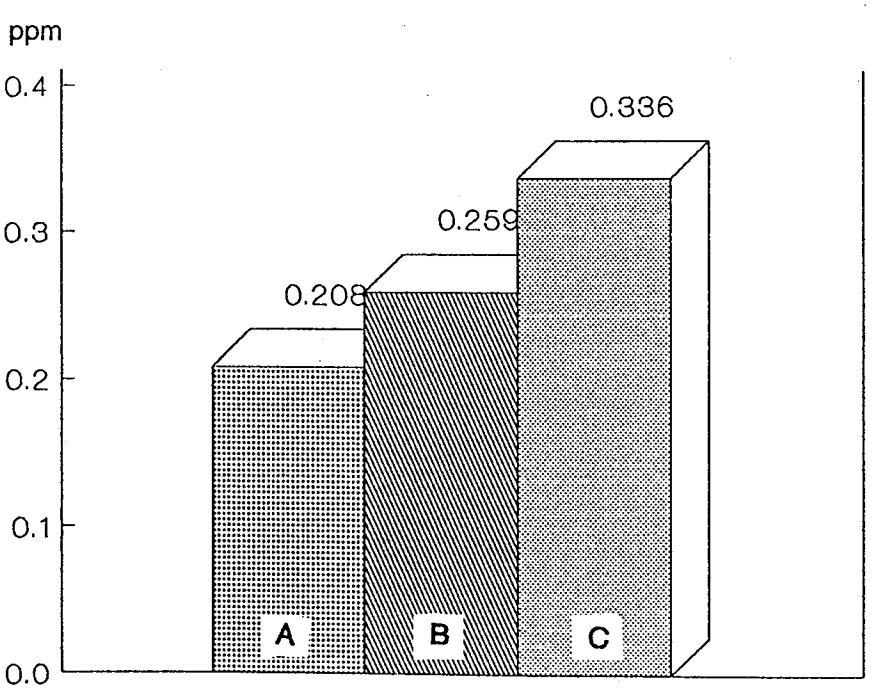
Values are Mean ± S.E.

TABLE 6. Catecholamine levels (μg/mL) in serum

Parameter	Control	Endemic Population
Adrenalin	220.67 ± 20.79	332.61 ± 20.54
Range	157.46 - 311.60	114.20 - 788.51
No. of cases	15	50
Nor-adrenalin	164.51 ± 11.19	514.87 ± 35.27
Range	118.51 - 235.0	108.64 - 1053.07
No. of cases	15	50

Values are Mean ± S.E.

FIGURE. Age dependent variation in serum fluoride concentrations among endemic population. A: below 30 yr. B: 30.- 50 yr. C: Over 50 yr.



Discussion

The study of health problems caused by water-borne fluoride in Mehsana and Banaskantha districts of North Gujarat revealed a wide occurrence of fluorosis, ranging from mild to acute.

The concentrations of fluoride ions in plasma is directly related to the fluoride content of the drinking water. This close relationship has been clearly demonstrated by several authors.[18,19] Saralakumari and Ramakrishna Rao[20] observed a correlation between fluoride toxicity and duration of stay in the village, nutritional and socio-economic status of the individuals.

In this study, when the individuals were divided into three age groups (under 30 yr, 30-50 yr, and over 50 yr), an increase in serum fluoride levels with age was observed (Figure). Ekstrand[21] showed that plasma fluoride increased with age between 10 and 38 years. This difference in serum fluoride levels could be attributed to a difference in fluoride uptake by the skeleton. The young, growing skeleton, being low in fluoride, has a greater capacity for taking it up. In older people, the bone fluoride is higher and the plasma approaches equilibrium with it, hence there occurs a rise in plasma fluoride with advancing years.[22] Experiments on dogs and puppies also revealed age-dependant increase in blood fluoride levels.[23] Thus there is a direct correlation between the age of the individual and fluoride retention.

There are only a few reports in the literature of anæmia in fluorotic individuals. Hæmoglobin levels in the endemic villages were low, compared to those in the control population. Though the difference between mean values was not significant, individual values in the endemic population showed great fluctuation. Though fluoride is capable of causing anæmia, the hæmoglobin level is also governed by the individual's nutritional status. Macuch *et al*[24] observed decreased hæmoglobin, increased erythrocytes and abnormal lymphocyte count in children living in the vicinity of an aluminium processing plant. Decreases in hæmoglobin and erythrocyte count were also reported in camels near a super-phosphate factory.[25]

Fluoride is known to inhibit protein synthesis, mainly due to impairment of peptide chain initiation[26] and by interfering with peptide chains on ribosomes.[27] In the present study protein levels in the endemic area were significantly decreased, which would adversely affect the growth of the affected individuals.

Conflicting reports have been published regarding fluoride toxicity and lipid metabolism. Saralakumari *et al* reported a decrease in plasma free fatty acids as well as total lipids, and an increase in serum cholesterol, in rats supplemented with fluoride in drinking water for sixty days,[28] but Chinoy *et al* showed no changes in serum cholesterol and various reproductive tissues of rats[2,6,7] and mice[29] exposed to NaF for 30 days. The results of the present investigation also revealed normal levels of serum cholesterol, thus ruling out the occurrence of hypo/hypercholesterolæmia among fluorotic individuals in the early stages of the disease.

The circulating levels of testosterone in fluorotic individuals were also not altered significantly in males. Chronic fluoride treatment of rats also resulted in decreased testicular cholesterol levels.[30]

Chronic cases of fluorosis need to be investigated in detail since the chances of atherosclerosis cannot be ruled out. Numerous investigators have reported calcification of arteries in association with skeletal fluorosis in high fluoride areas.[31,32] Susheela and Kharb have reported aortic calcification in rabbits subjected to chronic fluoride poisoning.[33] Fluoride also causes renal calcification in rats.[34] The decreased levels of serum calcium in the fluorotic individuals could be attributed to ectopic calcification in soft tissues. It could also be due to a decrease in the intestinal absorption of fluoride since fluoride is known to produce insoluble complexes with calcium. Further studies are necessary on endemic human populations to establish the role of fluoride atherosclerosis.

Calcium homeostasis is controlled by hormonal regulation of the thyroid and parathyroid glands.[35,36] In the present study, no significant change was observed in the levels of thyroid stimulating hormone (TSH) and T_3 in the fluorotic individuals. An enhancement was observed in the levels of T_4, which might be due to enhanced iodination to form this hormone rather than T_3 which would result in its increased synthesis/release by the gland. Demole did not find any relationship between thyroid dysfunction and fluoride toxicity.[37]

In fluorotic individuals the serum catecholamines were increased significantly, which would have a stimulatory effect on the sympathetic nervous system, thus influencing the hypothalamus gonadal axis and result in marked changes in reproductive functions. It would also affect the carbohydrate metabolism by accelerating the breakdown of glycogen.

The increased levels of serum transaminases in fluorotic individuals suggest alteration in liver function. These levels increase several times if cellular damage occurs in the liver, so these enzymes are markers for assessing liver function.

The fluorotic group showed marked alteration in their serum electrolyte levels. Potassium and sodium levels increased significantly, compared to controls. Differential distribution of these two cations is essential for normal membrane function and integrity. Similar augmentation in electrolyte levels was demonstrated in rats fed with sodium fluoride.[6] But Suketa and Terui observed a reduction in the serum sodium level in rats after fluoride administration, while potassium levels, on the other hand, significantly increased.[38] They attributed these changes to alteration in adrenal function. However, Das and Susheela reported low corticosteroid levels in fluorotic humans, suggesting adrenal hypofunction.[39] Serum potassium is an indicator of cell damage. Increased levels suggest cell deterioration.

Conclusion

The present study revealed wide occurrence of fluorosis in the Mehsana and Banaskantha districts of North Gujarat. Intake of high water-borne fluoride altered the normal body metabolism of the individuals. Further surveys are required in fluoride endemic areas, to reveal the magnitude of the problems caused by fluoride. The Government should take steps to supply safe drinking water in the villages affected by fluorosis.

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