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Occurrence of Endemic Fluorosis in Human Population of North Gujarat, India: Human Health Risk

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Excessive Fluoride intake in our body may result in a slow progressive, crippling scourge known as fluorosis. It was first detected in India, among cattle by the farmers of Andbra Pradesh during early 1930s (RGNDWM, 1993). Shortt et al. (1937) had published the first report on endemic fluorosis from India. It is estimated that about 25-30 million people in about 150 districts in India are suffering from varying grades of fluorosis.

Globally, it has been reported that apart from India, there are about 20 other developed and developing nations which. have come under the threat of fluorosis. These are Argentina, USA, Morocco, Algeria, Libya, Egypt, Jordan, Syria. Turkey, Iraq, Iran, Pakistan, Kenya, Tanzania, South Africa, China, Australia, New Zealand, Japan and Thailand.

In Indian subcontinent, a total of 15 states have been declared endemic for fluorosis. This does not rule out the possibility that even the remaining states and Union Territories are free from fluoride poisoning in the country (RGNDWM, 1993). Gujarat is one of the most severely affected state in the country considered to be endemic to fluorosis, where about 18 out of a total of 19 districts are prone to fluorosis due to high fluoride content in drinking water. Mehsana and Banaskantha districts located in North Gujarat and near to each other are considered to be the most affected districts apart from Amreli, Sabarkantha and Baroda (Barot,V.V.,1998).

In the present study, forty villages in Mehsana district and thirteen villages in Banaskantha district were surveyed and the effects of waterborne fluoride were investigated. Parallel studies were also conducted in Ahmedabad city (non-fluoride endemic area) population which was considered as control to compare the changes occurring due to consumption of high water borne fluoride in endemic population. For each individual, a detailed proforma sheet for health and water quality survey was filled up from each village, which included name,age,sex and address of the individual,food habbits,mottling of teeth,dental caries in premolars and canines as well as skeletal alterations etc.

MATERIALS AND METHODS

Fluoride levels in the drinking water and serum samples of control (Ahmedabad city) and human populations from endemic regions were estimated using ion selective electrode of Orion, U.S.A., Model 701A. Sodium and potassium levels from serum samples were analysed by using Perkin Elmer USA model 373 double beam atomic absorption spectrophotometer with background corrector system.

Blood glucose levels were estimated by the method of Nelson and Somogyi (1952). The catecholamine levels were estimated in blood serum using the method of Euler and Hamberg (1949). To 1 ml of the diluted serum, 1 ml of acetate buffer of pH 4 and 0.2 ml of iodine were added.

For epinephrine exactly after 1 and 1/2 minutes and for norepinephrine exactly after 3 minutes 0.5 ml of 0.05N. Sodium thiosulphate was added to it. Blank was also prepared using same buffer instead of iodine solution. Standards were run by using respective standard solutions. The color intensity of these samples was read on 106 calorimeter at 529 nm within 5 minutes.

The photometric determination of Serum Glutamate Pyruvate Transaminase (SGPT) and Serum Glutamate Oxaloacetate Transaminase (SGQT) were carried out by the method of Reitman and Frankel (1957). The serum was diluted 1:4 with physiological saline and used for estimation. However, SGOT assay consisted of L- aspartate instead of DL- alanine (phosphate buffer : 0.05M K_2HPO_4; 0.05M KH_2PO_4 0.1M L-aspartate; 0.002 M $\propto$ - ketoglutarate in 50 ml of double distilled water) and the tubes were incubated for exactly one hour after addition of serum.

The hormone concentration of the samples are quantitated by measuring radioactivity associated with the bound fraction of the samples or standards. Quantitative determination of T_3 and T_4 were carried out using radioimmunoassay reagent kits supplied by Bhabha Atomic Research Center (BARC), Mumbai, India. Thyroid stimulating hormone (TSH) assay was done using Immunoradiometric assay (IRMA) kit supplied by BARC. The assay of testosterone was carried out using kit standardized by Sereno laboratories, Italy. Follicle stimulating hormone (FSH) assay was carried out using diagnostic kit for the quantitative determination of serum FSH supplied by Binax laboratories, USA. For all the estimations a minimum of nine to ten replicates were taken and the data was subjected to statistical analysis using student's 't' test (Ispen and Feigl, 1970).

RESULTS AND DISCUSSION

In the present study, analysis of water samples collected from 15 different localities in Ahmedabad city showed a low concentration of 0.5 ppm fluoride to a maximum of 0.72 ppm with a mean of 0.64±0.013 ppm in water, while the survey conducted in 53 villages revealed wide variation in the levels of fluoride from a minimum of 1 ppm to a maximum of 6.53 ppm having a mean of 2.81±0.179ppm.

Bore water was more contaminated due to high fluoride content than well water (Table I).

Table 1. Showing the levels of fluoride in water and serum of control population and fluorotic human subjects of north Gujarat.

Group	Fluoride in Water (ppm)	Fluoride in Serum (ppm)
Ahmedabad City	0.638± 0.013	0.04± 0.002
Range	0.56-0.72	0.03-0.05
n	15	12
Endemic region	2.81± 0.179	0.278± 0.03
Range	1.00-6.53	0.131-0.552
n	53	76

Values are mean ± S.E.

Fluoride levels in the serum of fluoride afflicted individuals also varied considerably and a large number of people showed significantly high amount of fluoride as compared to control population, while low fluoride levels were also obtained in serum of some children and younger people but comparatively higher than the control population of Abmedabad city.

It has been reported that in areas with water containing less than 0.25 ppm of fluoride, the concentration of fluoride ions in blood was about 0.01 ppm (Murray et al., 1991).

Krishnamachari (1986) has correlated it with the degree of renal efficiency and reported of fluoride with other factors such as binders like Mg^{2+}, Ca^{2+}, Al^{3+} etc. Similarly, Murray et al (199 1) have reported that uptake by the skeleton particularly in growing children which is low in fluoride has a greater capacity. While in older people, the bone fluoride is higher and it approaches equillibrium in plasma, where it rises with advancing years.

Na^{+} and K^{+} levels in serum showed significant increase due to consumption of higher levels of fluoride in drinking water in case of fluorotic population as compared to the control population of Ahmedabad city (Table 2). The rise in these levels could be attributed to the alterations in electrolyte balance in inter or/and intracellular fluids as well as Na^{+} and K^{+} levels consumed in drinking water. As a result, this might influence the movement of water inside as well as outside the cells. The overall distribution of these two cations is vital in many membrane systems where energy oriented active transport is functional. It has been reported that fluoride is known to cause potassium efflux from cells (Mclvor et al., 1985).

Table 2. Showing the levels of different biochemical estimations in the sera of both the groups of human population.

Sr. No.	Parameter	Control Population	Fluorotic Population
1.	Sodium (ppm) Range n	1163.68± 28.84 861-1500 22	1875.59 ± 30.8 1320-2450 59
2.	Potassium (ppm) Range n	129.35±7.96 90-210 20	322.75±42.38 130-820 59
3.	Blood glucose (mg/100ml) Range n	136.32±5.94 108.88-168.88 10	89.44±6.98 48.96-175.77 25
4.	Epinephrine(μg/ml) Range n	220.67±20.79 157.46-311.69 11	332.61±20.54 114.2-788.51 45
5.	Nor-epinephrine (μg/ml) Range n	164.51±11.19 118.51-235 11	514.87±35.27 108.64-1053.07 45
6.	SGOT (mU/ml) Range n	16.05±1.2 12.00-24.00 10	29.38±0.83 23.00-41.00 30
7.	SGPT (mU/ml) Range n	11.70±0.83 9-15 10	22.33±0.73 15-29 30

Values are mean ± SE.

Despite the fact that fluoride is a potent toxicant which disturbs carbohydrate metabolism, yet the precise mechanism of its action is still not clearly understood.

The results in the present study revealed a significant decline in the levels of blood glucose in fluoride afflicted human population of North Gujarat as compared to control (Table 2).

Several studies from our laboratory (Chinoy et al.,1993,Chinoy and Sequeira, 1989) have confirmed accumulation of glycogen in liver, gastrocnemius and pectoral muscles, vas deferens and uterus of experimental mice and rats. Such type of accumulation would indicate low carbohydrate utilization and energy

turnover in respective tissues. This could be correlated with the levels of blood glucose.

On the contrary, an increase in the circulating glucose levels in the mudskipper (Boleophthalmus dussumieri) was reported by Shaikh and Hiradhar (1985) and in flnoride intoxicated rats resulting in marked hyperglycemia (McGown and Suttie, 1977).

Similarly, the serum epinephrine and nor-epinephrine levels were increased significantly. This might be due to the stress on adrenal gland or else enhanced release of these hormones due to accumulation of fluoride in body (Patel et al., 1994). This in turn affected the feedback control mechanism. The enhanced levels of catecholamines would probably also influence the hypothalamo - gonadal axis.

Earlier workers (Cheon and Distefano, 1973) have demonstrated that fluoride significantly increased the catecholamine levels of the liver, heart and kidney. Similar findings were also obtained in sodium fluoride treated female mice from our laboratory (Pate1 et al., 1994).

Thus, these elevated catecholamines would alter the carbohydrate metabolism which was correlated with the glucose concentration due to fluoride toxicity in laboratory rodents (Dost et al., 1977).

Liver is known to be severely affected by fluoride toxicity. The activities of serum transaminases (i.e. SGOT and SGPT) were increased significantly in fluorotic population in endemic regions as compared to control.

It is known that these enzymes are the markers for liver function and in the event of liver cell damage, SGOT and SGPT would be released in excess.

In agreement with these results, augmented liver GOT and GPT activities by fluoride exposure in fish (Chitra et al.,1983) and in goats (Tsunoda et al., 1985) were reported.

Similar findings were also reported in animal models and endemic populations indicating alterations in liver function (Chinoy et al., 1994).

The levels of serum triiodothyronine (T_3), thyroxin (T_4), Follicle Stimulating Hormone (FSH), Thyroid Stimulating Hormone (TSH) and testosterone are summarized in Table 3. In this study, people residing in fluoride endemic regions did not exhibit any significant alteration in T_3 and TSH levels, while T_4 levels were comparatively enhanced as compared to the population in Ahmedabad city. An increase in the levels of serum thyroxin might be due to its enhanced synthesis or decline in its utilization.

As a result, the basal metabolic rate (BMR) might be affected which would be responsible for altered cellular activities. However, Sonneborn and Mandelkow (1981) suggested that there is no relationship between goiter and fluoride toxicity.

Table 3. Showing the comparative data on hormonal profile of both the groups of human population.

Sr. No.	Hormone Type	Control Population	Fluorotic Population
1	T_3 (μg/ml)	1.50±0.135	1.53 ± 0.076
	Range	0.70-2.10	1.00-3.70
	n	14	40
2.	T_4 (μg/dl)	9.16±0.63	14.77±0.512
	Range	5.40-13.00	7.20-20
	n	14	40
3.	TSH (μU/ml)	2.56±0.36	2.55±0.37
	Range	0.50-4.40	7.20-20
	n	14	40
4.	FSH (mIU/ml)	10.87±1.13	13.39±2.05
	Range	2.10-18.20	3.80-25.30
	n	15	44
5.	Testosterone (ng/ml)	6.42±0.423	5.56±0.489
	Range	4.30-9.30	0.64-11.50
	n	11	29

Values are mean ± S.E.

FSH is known to stimulate the activities of gonads. In these investigations, FSH activities were not altered significantly in the afflicted individuals. These results elucidate that folliculogenesis might not be affected, and in male individuals. spermatogenesis is likely to be maintained (Li et al.,1987). Serum testosterone levels indicated significant decline in case of fluorotic individuals. This might be due to impaired steroidogenesis or else alteration in hormone receptor interaction. Since it is known that phospholipids especially phosphatidylinositol (PI) which is involved in hormone receptor action was reduced in testis and epididymis.

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