STUDIES ON EFFECTS OF FLUORIDE IN 36 VILLAGES OF MEHSANA DISTRICT, NORTH GUJARAT

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SUMMARY: A survey was carried out in 36 fluoride endemic villages of Mehsana District of North Gujarat. Urine and blood samples of fluoride-afflicted human population and their drinking water were analysed for fluoride content and compared with samples from different parts of Ahmedabad city (control). The fluoride content in water samples of Ahmedabad city was within the permissible limits, but was high in endemic villages. The urine and serum of individuals from these villages also showed a higher concentration of fluoride than in the control population. The enhanced Na^+ and K^+ levels in the urine of the fluorotic populations indicates a probable electrolyte imbalance and altered kidney functions. Similarly, higher activities of serum transaminases (SGOT and SGPT) might be due to altered liver function, since both of these enzymes are known markers (of liver function). Normal steroidogenesis in fluorotic subjects was evident by the unaffected serum testosterone levels. Serum cholesterol was also in the normal range which indicates that fluorotic subjects were not suffering from hypercholesterolemia. Serum sialic acid, a known marker for detection of fluorosis, was reduced in cases from endemic villages. This might be due to escalated concentration of glycosaminoglycans, which hinder hormone-receptor interaction. Thus, the above data reveal altered liver and kidney function in fluorosis-afflicted individuals with high urine and serum fluoride but low sialic acid levels compared to normal controls.

Key words: Fluoride; Human population; Mehsana; Survey study.

Introduction

Fluoride is ubiquitously distributed in the soil, water, food and air. Water is ordinarily the principal medium of fluoride intake by the human population. Excessive concentrations of fluoride in drinking water lead to crippling fluorosis in endemic areas. More than a million people in India are afflicted with skeletal and dental fluorosis. The fluoride absorbed through the gastrointestinal tract is rapidly distributed to all the tissues by simple diffusion. Fluorine, the most electronegative element, can rapidly cross the cell membrane, skeletal and cardiac muscle, liver, skin (1) and the erythrocytes (2). Even placental transfer of fluoride by diffusion is known which can impose deleterious effects on foetal development (3). Under certain conditions, the absorbed fluoride can affect virtually every phase of human metabolism.

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Endemic fluorosis is prevalent in 15 states in India where, due to the hot climate, large quantities of water containing comparatively high levels of fluoride are drunk (4). Gujarat is one of the 15 states where fluorosis occurs in five districts. A large faction of population, especially of economically backward classes, were found highly susceptible to the disease. A survey was therefore carried out in 36 villages of Mehsana district of Gujarat state. Drinking water samples, along with blood and urine, were collected to study the alterations brought about by fluoride ingestion in these individuals.

Materials and Methods

Thirty six villages located in the Mehsana district of North Gujarat were suveyed. The inhabitants of these villages, prior to the collection of urine and blood samples, were checked for apparent mottled teeth, back pain, stiffness of back and joints and other abnormalities including skeletal problems. Most of these individuals were unable to bend due to a stiff back, which indicated that they were afflicted with fluorosis. The details of each case and the source of drinking water were recorded in the proforma sheet. A total of 210 samples of urine and 68 samples of blood were obtained. The blood was transferred to culture tubes after collection by using hypodermic syringe and allowed to clot fully, and the serum was separated by centrifugation. The urine samples were collected in clean, dry plastic bottles. Immediately after collection, 2 to 3 drops of toluene were added to prevent fungal growth. Drinking water samples from all these villages were also collected. Blood, urine and water samples from inhabitants of Ahmedabad city and its vicinity were collected and used as controls for various biochemical parameters.

Fluoride content in water, urine and serum

Fluoride concentrations (expressed in ppm) in water, urine and serum samples were determined with an Ion Selective Electrode Orion Model 701A.

SGOT and SGPT

The photometric determinations of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were carried out by the method of Reitman and Frankel (5). A 0.2 ml sample of diluted serum (1:2) was incubated at 37° C with 1 ml of buffered solution (pH 7.4) (α-ketoglutaric acid and aspartic acid for SGOT; α-ketoglutaric acid and alanine for SGPT). One ml of colouring reagent was added and kept at room temperature for 20 minutes. To these, 10 ml of 0.4 N NaOH was added. The respective quantities of oxaloacetate and pyruvate formed were measured in a Bausch and Lomb Spectronic 88 Colorimeter at 546 nm and expressed as mU/ml.

Cholesterol

Cholesterol concentration in serum was determined by the method of Pearson *et al* (6). To 5 ml of colouring reagent (para-toluenesulphonic acid in glacial acetic acid and acetic anhydride 40:60 respectively), 0.2 ml of serum and 1 ml of concentrated sulphuric acid (H_2SO_4) was added. Cholesterol reacts at room temperature with acetic anhydride and H_2SO_4 gives an intense brown-red complex, which was measured in Spectronic Colorimeter 106 at 620 nm and expressed as mg/100 ml serum.

Testosterone

The levels of testosterone in serum were assayed by using radioimmunoassay (Double antibody technique) of Peterson and Swerdloff (7) with reagents standardised in RIA kit supplied by M/s Serono Laboratories, Italy. An unlabelled hormone of unknown concentration in the standard (sample) competes with a known concentration of radiolabelled hormone for the limited binding sites of the specific antibodies. At the end of the incubation, the antibody-bound and free hormone were separated by the addition of second antibody, after which precipitation occured. The pellet was then counted by placing each tube for one minute in a Beckman Automatic Gamma Counter (Model 5500). The hormone concentrations of the samples were quantitated by measuring the radioactivity associated with the bound particles of the samples or standards and expressed as ng/ml.

Sialic acid

Sialic acid concentrations in serum were determined by the method of Jourdian *et al* (8). To 0.5 ml of diluted serum (1:10), 0.1 ml of periodic acid (0.04 M) was added and kept in an icebath for 20 minutes. To these was added 1.25 ml of resorcinol (0.6%) and the solutions boiled at 100° C for 15 minutes, cooled and then 1.25 ml of t-butynol alcohol was added. The periodate oxidation of glucosidically bound sialic acid gives a chromogen, which reacts with resorcinol. The colour intensity was measured in a Spectronic 106 Colorimeter at 630 nm and expressed as μg/ml serum.

Urinary Na^+, K^+

The sodium and potassium levels of urine were estimated on a Systronics Flame Photometer, Digital Unit Type 125, by the method of Dean (9) and expressed as ppm. Solutions of NaCl and KCl (1 to 9 ppm) were used as standards. Diluted urine for analysis was sprayed as a fine mist into a non-luminous flame, which becomes coloured according to the characteristic emission of the metal. A narrow band of wavelength corresponding to the element being analysed was selected by a light filter and allowed to fall on a photodetector, which was the concentration of the element measured in the digital display.

Statistics

A minimum of 20 replicates were taken for each parameter and the data were statistically analysed using Student's 't' test.

Results

Fluoride in water

Fluoride content in water was significantly higher ($p < 0.001$) in Mehsana district, a fluoride endemic area, compared to the non-endemic areas selected from Ahmedabad city and its vicinity (Table 1).

Fluoride in urine

Fluoride level was enhanced significantly ($p < 0.001$) in the urine of fluorotic individuals compared to controls (Table 1).

Fluoride in serum

Serum fluoride concentration was also increased significantly ($p < 0.001$) in the fluorotic human population of Mehsana district in comparison to controls (Table 2).

Table 1
Fluoride concentration in water and urine of control and fluoride endemic population

Area	Water Fluoride (ppm)	Urine Fluoride (ppm)
Control (Ahmedabad)	0.62 ± 0.02	0.67 ± 0.01
Range	0.6 - 1.04	0.1 - 1.5
n	24	50
Endemic Region (Mehsana District)	2.2 ± 0.05	4.2 ± 0.5
Range	1.5 - 3.9	1.4 - 8.9
n	36	210

Values are mean ±S.E.
n = number of samples

Table 2
Fluoride concentration in serum in control and endemic population

Area	Fluoride (ppm)
Control (Ahmedabad)	0.04 ± 0.002
Range	0.03 - 0.05
n	25
Endemic Region (Mehsana District)	0.32 ± 0.04
Range	0.18 - 0.79
n	68

Values are mean ±S.E.
n = number of samples

Table 3
Activities of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGOT) in control and endemic population

Area	SGOT (mU/ml)	SGPT (mU/ml)
Control (Ahmedabad)	15 ± 0.80	10 ± 0.84
Range	12.5 - 20	6.5 - 13.5
n	25	25
Endemic Region (Mehsana District)	58 ± 4	56 ± 6
Range	14 - 86	16 -120
n	68	68

Values are mean ±S.E.
n = number of samples

Table 4
Serum cholesterol and testosterone levels in control and endemic populations

Area	Cholesterol (mg/100ml)	Testosterone (ng/ml)
Control (Ahmedabad)	184 ± 5.0	6.4 ± 0.42
Range	150 - 195	3 - 9
n	25	25
Endemic Region (Mehsana District)	190 ± 5	6.38 ± 0.45
Range	153 - 220	2.85 - 8.8
n	68	68

Values are mean ±S.E.
n = number of samples

Table 5
Serum sialic acid concentration in control and endemic population

Area	Sialic acid (μg/ml)
Control (Ahmedabad)	200 ± 0.88
Range	180 - 236
n	25
Endemic Region (Mehsana District)	168 ± 4
Range	114 - 223
n	68

Values are mean ±S.E.
n = number of samples

Table 6
Na^+, K^+ levels in urine of control and endemic populations

Area	Na^+ (ppm)	K^+ (ppm)
Control (Ahmedabad)	2850 ± 314	1670 ± 265
Range	1300 - 4000	600 - 3000
n	50	50
Endemic Region (Mehsana District)	3567 ± 272	2541 ± 227
Range	1200 - 7900	750 - 5500
n	210	210

Values are mean ±S.E.
n = number of samples

Serum SGOT and SGPT

The activities of SGOT and SGPT in the endemic populations increased significantly ($p < 0.001$) compared to controls (Table 3).

Serum cholesterol

Cholesterol in serum showed no alterations in fluoride-afflicted individuals in comparison to controls (Table 4).

Serum testosterone

Serum testosterone levels were also unaffected in fluorotic individuals compared to controls (Table 4).

Serum sialic acid

The concentration of sialic acid was decreased significantly ($p < 0.01$) in the fluorotic populations in comparison to the control population (Table 5).

Urinary Na^+, K^+

The Na^+ and K^+ levels in urine of fluoride afflicted individuals showed a significant increase ($p < 0.001$) compared to controls (Table 6).

Discussion

The fluoride content in drinking water of the endemic villages was higher than the permissible level of 1 ppm, according to WHO (10). Serum and urinary fluoride concentrations were also significantly higher in the fluorotic subjects of these areas. Extensive literature shows that fluoride, even at very low concentrations, inhibits several enzymes involved in various metabolic processes of the body (11,12). In the present study the high fluoride content in urine and serum due to greater intake through water probably adversely affects the general body metabolism in the fluorotic cases.

Renal tissues are highly sensitive to fluoride, which thereby causes damage to the kidneys. As the damage increases, clearance of fluoride decreases (13). The toxic effects of fluoride are aggravated by the altered clearance of electrolytes. Thus, the rise in Na^+ and K^+ levels of urine could be attributed to changes in electrolyte balances in intercellular and intracellular fluids, which in turn may influence the movement of water in and out of the cellular matrix. The differential distribution of these two cations is essential in many membrane systems, where energy requiring active transport is functional. Fluoride in excess in the intracellular region results in Na^+ influx and K^+ efflux (14). The altered ionic concentrations might result in dysfunction of aldosterone action at selective resorption sites in kidney, which can cause the decrease in body weight due to loss of water along with the salts. Suketa and Terui (15) also reported that altered Na^+ and K^+ levels in urine and serum of fluoride-intoxicated rats might be due to a disturbance of adrenal function. Therefore, changes in Na^+ and K^+ levels in the urine of fluorotic subjects might cause altered adrenal function in these cases.

The activities of SGOT and SGPT are known markers of liver function. The activities of both these transaminases were significantly increased in the fluorotic human beings, which would indicate the hepatocellular death or damage and changes in liver function (16) in the fluorotic human population. Triggered activity of these transaminases and liver damage following the ingestion of fluoride in different species of animals have also been reported (17,18).

In the present study, serum cholesterol and testosterone levels of fluoride afflicted human subjects were found to be unaffected. This observation suggests unaltered steroidogenesis in these individuals. Similar findings were also obtained in rodents (mice, rabbits and rats) by fluoride ingestion. The Leydig cell morphology was also unchanged (19,20). Despite unaltered cholesterol and testosterone levels in fluoride ingested rodent models, various androgen-dependant parameters in different target organs were adversely affected, especially cauda epididymis leading to dysfunction of sperm and thus contributing towards low fertility. In corroboration of these results, recently Neelam *et al* (21) reported prevalence of infertility among residents in high fluoride endemic areas in Andhra Pradesh, India. This might be due to alterations in the conversion of testosterone into its potent metabolite, 5α-dihydrotestosterone and the enzyme 5α-reductase; or to impaired hormone-receptor interaction and consequent impaired target organ response due to increase in prostaglandins E2 and PGF2 by fluoride (22). Both these PGs are known to manifest antiandrogen effects on the male reproductive system (23,24). Hence, further studies in this direction are under way at present.

In the serum of fluorotic human subjects, sialic acid concentration decreased significantly. Susheela and Jha (25) and Jha *et al* (26) also reported decreased sialic acid and increased glycosaminoglycans in the serum of fluorotic subjects and have suggested that sialic acid concentration is a marker for detection of fluorosis. Sialic acid, a sialomucoprotein, maintains equilibrium with its derivatives, the glycosaminoglycans, in the serum. The latter are known to attach themselves to exogenous proteins of the plasma membrane by glycosidic and glucosidic bonds and thus play an important role in the interaction of hormone-membrane bound receptor molecules. Hence, the above mentioned alterations in sialic acid might cause disturbance in hormone action at the target cell in fluorotic cases.

From the above data, it is essential to investigate a therapeutic agent that could effectively ameliorate the effects of fluoride in the endemic population, and is easily available and cheap. The role of ascorbic acid and calcium individually and in combination in the mitigation of fluoride effects has been reported elsewhere (27). The role of vitamin C in the mitigation of fluoride effects has been investigated by Venkateswarlu and Narayana Rao (28). Therefore, in view of our animal data, studies on the ameliorative role of ascorbic acid and calcium individually and/or in combination are called for to help relieve the suffering of millions afflicted with fluorosis.

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