Studies on the Biological Effects of Fluoride Intoxication in Dental Fluorosis Cases

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Abstract: Dental fluorosis, a mundane problem, continues as a permanent health related issue in the modern days also. India is not an exception to this age old human menace. The present study tries to find out the biological effects of fluoride intoxication in dental fluorosis cases in Kanyakumari District, Tamil Nadu, the southern land mark of Indian sub-continent. There is a direct influence of fluoride on certain specific biomarkers. The inhibitory role of fluoride on some of the hydrolytic enzymes such as cholinesterase (73% in males, 70% in females), lactate dehydrogenase (58.6% in males, 57.7% in females) and acid phosphatase (45.9% in males, 32.1% in females), hormones like testosterone (55.9% in males, 42.1% in females), prolactin (32% in males, 82.6% in females), free triiodothyronine (50.4% in males, 43.8% in females) and free thyroxine (51.8% in males, 46.5% in females) are evidenced when compared to their respective control groups. The excitatory role of fluoride on the enzyme alkaline phosphatase (182.5% in males, 55% in females) and hormones like follicle stimulating hormone (94% in males, 142% in females) and thyroid stimulating hormone (489% in males, 343% in females) are noticed. A linear relationship between the severity of dental fluorosis (df) and the urinary as well as the serum fluoride concentrations and the age related increase of serum fluoride in the df cases are discussed.

Key words: Age old; Biomarkers; Dental fluorosis; India; Inhibitory/ excitatory role; Kanyakumari District; Mundane problem; Serum fluoride; Tamil Nadu; Urinary fluoride.

INTRODUCTION

“Dental fluorosis”, or “mottled enamel”, a unique water-borne disease, is one of the most crippling diseases afflicting millions of people in most parts of the world. This age old menace is not new to India and it continues as one of the major public health problems. Fluorosis is endemic in many Indian states. (Teotia and Teotia, 1984; Susheela, 1993) There are 62 million affected people, including 6 million children in 18 of 33 constituent States and Union territories of India. (Susheela, 1999)

Fluorosis endemic districts of Tamil Nadu are coming under the category III, where more than 50 percent of the districts are found as fluorosis hyper-endemic areas. (Susheela, 991) Mottled dental enamel and diffuse osteosclerosis of the skeleton was first described in India from the state of Madras by Shortt et al. (1937) Subsequently cases have been described from other parts of India by many researchers, (Pandit et al., 1940; Daver, 1945; Siddiqui, 1955; Singh et al., 1963; Teotia et al., 1939) and from other countries where the drinking water contains excessive quantities of natural fluoride. (Dean, 1933; Zipkin et al., 1958; Azhar et al., 1961).

Biological effects of fluoride intoxication capable of causing drastic changes in the cellular and blood components are of prime concern in man. Metabolic changes by fluoride have been reported on soft tissues like thyroid, reproductive organs, liver, kidney, brain and adrenal glands. (Mysliwiec et al., 2002) The present study is carried out to elucidate the relative role of fluorosis on various biological agents in the serum of diseased persons in the selected rural areas (Azhagappapuram, Lakshmipuram and Punnarkulam) in Kanyakumari district, Tamil Nadu, India. Fluoride concentration in the urine and serum samples of the victims are also tested to find out the fluoride intoxication.

MATERIALS AND METHODS

To assess the magnitude of dental, skeletal and non-skeletal fluorosis, house to house survey was

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conducted and the clinical examinations were performed by the assistance of experienced dentists. The survey covered people from 8-60 plus years of age, which includes children and adults of both sexes. Dental fluorosis was assessed by Dean’s classification system, Dean, (1942) which categorises the survey group into normal, questionable, very mild, mild, moderate and severe cases.

Standard methods were used for various biochemical estimations such as acid phosphatase (ACP), Bergmeyer, (1984) alkaline phosphatase (ALP), Tietz, (1999) lactate dehydrogenase (LDH), Young, (1997) cholinesterase (ChE), King, (1974) follicle stimulating hormone (FSH), Lenton et al., (1982) testosterone, Lenton et al., (1982) prolactin, Lenton et al., (1982) thyroid stimulating hormone (TSH), Beck-Peccoz and Persani (1994) free triiodothyroine (fT3), Wild, (1994) and free thyroxine (fT4), Midgeley John., (2001) in serum. Urine samples were collected in plastic bottles on the spot and tested for the fluoride content by the method described, Hall et al., (1972). Blood samples were also collected in plastic bottles, serum was separated and tested for fluoride by the method of Hall et al. (1972).

RESULTS AND DISCUSSION

Table 1 shows some of the important hydrolytic enzyme activities in the serum samples of the severe fluorotic adult cases. The mean ± SD values of ACP; ALP, ChE and LDH in the diseased males and females are 1.8 ± 0.4, 4.3 ± 0.458; 564.7 ± 14.629, 289.9 ± 14.293; 1739.5 ± 137.758, 1800.7 ± 116.087; 167.9 ± 10.802, 170.8 ± 7.666 respectively.

Table 1: Reveals the serum hydrolytic enzyme activities in dental fluorosis (severe) cases.
(sample, n=20 subjects: 10 men + 10 women; control, n=6 subjects: 3 men + 3 women)
(Values are mean ± SD)

<table>
<thead>
<tr>
<th>Name of the enzyme(s)</th>
<th>Name of the sample(s)</th>
<th>Enzyme activity (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid phosphatase</td>
<td>a) endemic normal</td>
<td>3.33 ± 0.471</td>
</tr>
<tr>
<td></td>
<td>b) df cases</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>a) endemic normal</td>
<td>200 ± 21.602</td>
</tr>
<tr>
<td></td>
<td>b) df cases</td>
<td>564.7 ± 14.629</td>
</tr>
<tr>
<td>Cholinesterase</td>
<td>a) endemic normal</td>
<td>6441 ± 660.005</td>
</tr>
<tr>
<td></td>
<td>b) df cases</td>
<td>1739.5 ± 137.758</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>a) endemic normal</td>
<td>406 ± 2.16</td>
</tr>
<tr>
<td></td>
<td>b) df cases</td>
<td>167.9 ± 10.802</td>
</tr>
</tbody>
</table>

Results for the activities of certain important hormones such as FSH, testosterone, prolactin, TSH, fT3 and fT4 in the adult male and female fluorotic cases show deviation from the control groups (Table 2). An enhanced level of FSH activity is detected in the diseased groups (i.e) 94% in males and 142% in females compared to their respective control. Similarly, an elevated level of TSH activity is noticed (i.e) 5.9 folds in male and 4.4 folds in female df cases.

Table 2: indicates the hormonal activities in the serum of dental fluorosis (severe) cases.
(df severe cases, n=20 subjects include 10 men + 10 women; control, n=6 subjects include 3 men +3 women)
(Values are mean ± SD)

<table>
<thead>
<tr>
<th>Name of the hormone(s)</th>
<th>Name of the sample (s)</th>
<th>Hormonal activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH</td>
<td>a) endemic normal</td>
<td>7.36 ± 1.317</td>
</tr>
<tr>
<td></td>
<td>b) df cases</td>
<td>14.27 ± 1.776</td>
</tr>
<tr>
<td>Testosterone</td>
<td>a) endemic normal</td>
<td>6.99 ± 0.109</td>
</tr>
<tr>
<td></td>
<td>b) df cases</td>
<td>3.08 ± 0.072</td>
</tr>
<tr>
<td>Prolactin</td>
<td>a) endemic normal</td>
<td>6.96 ± 0.123</td>
</tr>
<tr>
<td></td>
<td>b) df cases</td>
<td>4.73 ± 0.360</td>
</tr>
<tr>
<td>TSH</td>
<td>a) endemic normal</td>
<td>0.99 ± 0.066</td>
</tr>
<tr>
<td></td>
<td>b) df cases</td>
<td>5.83 ± 0.110</td>
</tr>
<tr>
<td>fT3</td>
<td>a) endemic normal</td>
<td>3.67 ± 0.115</td>
</tr>
<tr>
<td></td>
<td>b) df cases</td>
<td>1.82 ± 0.106</td>
</tr>
<tr>
<td>fT4</td>
<td>a) endemic normal</td>
<td>1.14 ± 0.165</td>
</tr>
<tr>
<td></td>
<td>b) df cases</td>
<td>0.55 ± 0.031</td>
</tr>
</tbody>
</table>

The activities of testosterone, prolactin, fT3 and fT4, seem to be lower in the severe fluorotic adult subjects of both sexes than that of their respective endemic normal groups. Testosterone activity is reduced 56% in males and 42% in females. There is an abrupt reduction of prolactin activity also (i.e) 32% in males and 83%
in females. Results also show a reduction of 50% fT₃ activity in males and 48% in females; 56% fT₄ in males and 46% in females.

Table 3: reveals the age of puberty and fluoride levels in urine and serum samples of fluorotic girls. (n=363 fluorosis cases; control, n = 3 individuals in each age group) (Values are mean ± SD)

<table>
<thead>
<tr>
<th>Puberty age (Yrs)</th>
<th>No. of subjects</th>
<th>Fluoride content (mg/L)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine</td>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>Df cases</td>
<td>Normal</td>
<td>Df cases</td>
</tr>
<tr>
<td>8</td>
<td>5.2 ± 0.01</td>
<td>7.2 ± 0.2</td>
<td>0.3 ± 0</td>
<td>1.61 ± 0.1</td>
</tr>
<tr>
<td>9</td>
<td>5.3 ± 0.2</td>
<td>7.6 ± 0.5</td>
<td>0.3 ± 0.01</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>10</td>
<td>5.7 ± 0.3</td>
<td>8.2 ± 0.3</td>
<td>0.3 ± 0</td>
<td>1.71 ± 0.1</td>
</tr>
<tr>
<td>11</td>
<td>5.9 ± 0.4</td>
<td>8.8 ± 0.4</td>
<td>0.3 ± 0.02</td>
<td>1.73 ± 0.2</td>
</tr>
<tr>
<td>12</td>
<td>5.8 ± 0.5</td>
<td>9.9 ± 0.5</td>
<td>0.3 ± 0</td>
<td>1.76 ± 0.1</td>
</tr>
<tr>
<td>13</td>
<td>5.82 ± 0.4</td>
<td>9.3 ± 0.6</td>
<td>0.3 ± 0.2</td>
<td>1.79 ± 0.2</td>
</tr>
<tr>
<td>14</td>
<td>5.92 ± 0.3</td>
<td>9.1 ± 0.4</td>
<td>0.3 ± 0.1</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>15</td>
<td>5.96 ± 0.2</td>
<td>9.4 ± 0.5</td>
<td>0.3 ± 0.2</td>
<td>1.82 ± 0.1</td>
</tr>
<tr>
<td>16</td>
<td>5.98 ± 0.1</td>
<td>9.7 ± 0.3</td>
<td>0.3 ± 0.1</td>
<td>1.91 ± 0.1</td>
</tr>
</tbody>
</table>

Correlation: Normal Vs df cases
For urine: r = 0.89; for serum, r = 0.

Results in table 3 reveals the age of puberty and the fluoride contents in the urine and serum samples of the fluorotic girls. The puberty age of the 363 girls falls inbetween 8-16 years. The mean fluoride levels in the urine samples of the control groups range between 5.2-5.98 mg/L, and it is 7.2-9.7 mg/L in the fluorotic subjects. But, irrespective of the puberty age, the mean serum fluoride levels of the control groups are 0.3 mg/L, where as it is 1.61-1.91 mg/L in the fluorotic subjects.

Fig. 1: shows the fluoride levels in the urine samples of the selected normal and df cases

Fig. 2: shows the fluoride levels in the serum samples of the selected normal and df cases
The mean fluoride levels in the urine samples of the selected normal and fluorotic subjects (20 cases in each category) are depicted in figure 1. Fluoride levels in various categories of the male subjects are noticed in between 5.2-9.1 mg/L, whereas in females it is 5.1-8.9 mg/L. Figure 2 shows the mean serum fluoride values of the selected normal and fluorotic subjects (20 cases in each category). It ranges between 0.2-0.4 mg/L in both males and females. There is a positive correlation for the urine versus serum samples of both males ($r=0.804$) and females ($r=0.813$) with a $p$ value 0.0002.

Discussion:

As an enzyme inhibitor, there are contradictory reports on the action of fluoride on enzymes and hormones. Earlier reports reveal significantly higher levels of ALP, (Swarup et al., 1998; Gupta et al., 1994; Farly et al., 1983; Shivashankara et al., 2000) and lower levels of cholinesterase, (Vanaja Paul et al., 1998; Ekambaram and Vanaja Paul, 2001) LDH, (Kenji Akiniwa, 1997; Hanaa and Malhotra, 2009) and ACP, Webb - peploe and Bradley, (1996) in fluorotic subjects. Our study also supports this view. The present study finds an elevated level of ALP activity. Reduced activity of cholinesterase in the df cases may delay the nerve impulse transmission, reduction of this neurotransmitter may produce insensitivity, tingling and numbing in the extremities, the common problems in the diseased ones. Lack of extensive and large scale studies on the action of fluoride on enzymes in man is the lacuna in fluorosis research.

A significant increase of FSH and a decrease of testosterone and prolactin activities in our fluorotic cases indicates that fluoride toxicity may cause serious effects on the reproductive systems in fluorotic cases. Our work supports the previous reports on the fluoride induced effects in experimental models and humans, (Susheela and Jethanandani, 1996; Ortix - Perez et al., 2004).

The enhanced activity of TSH and reduced activities of fT3 and fT4 in the present study also coincides with the previous studies, (Mc Laren, 1976; Bobek et al., 1976; Xiaoli et al., 1999). The abnormal levels of thyroid hormones in the df cases is an indication of malfunctioning of the thyroid gland by fluoride. It is an alarming call to take immediate steps to restore the health conditions of people in fluorosis endemic areas.

High urinary fluoride levels, Gupta et al., (1994) in df cases are the best indicator of heavy fluoride intake. (Dinman et al., 1976a; Reddy et al., 1998) A linear increase of urinary fluoride level with respect to puberty age in girls ($r=0.89$) is observed between the diseased versus the normal ones. Serum fluoride concentrations is recognised as a good indicator of fluoride exposure and provides useful data for endemic fluorosis control and prevention, (Wan et al., 2002; Kono et al., 1993; Li and Ke, 1990). Puberty age versus serum fluoride levels in df cases shows a positive relationship with ‘0’ correlation.

Figure 1 and 2 illustrates the linear relationship between the severity of the infection and the urinary and serum fluoride concentrations. The study present also supports the work of Torra et. al., (1998) where serum fluoride concentration is related to age and not to sex.

The present findings reveal that, fluoride inhibits some of the enzymes and hormones drastically in males than the fluorotic females (except FSH and prolactin). The contradictory effect of fluoride on enzymes and hormones came to light only after the quantitative estimation of these biomarkers. Enhanced activities of ALP and TSH in men and FSH and prolactin in women are very clear from this study. These findings imply that, males are highly susceptible to fluoride than the females. However, it should be confirmed with further large scale studies.

Fluoride threw normal life out of gear by disturbing the normal metabolic activities via enzyme and hormonal systems. Metabolic irregularities in the diseased make them disabled, causing loss of man power and economic potential. Better prophylaxis will provide proper health-care awareness and the supply of safe potable water in the fluorosis endemic areas will safeguard the present and future generations. The study present paves a way to understand the excitatory / inhibitory roles of fluoride on various enzymes and hormones in the diseased, and also highlights the excessive presence of fluoride in urine and serum.

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