Research Article

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Effect of excess Fluoride consumption on Urine-Serum Fluorides, Dental state and Thyroid Hormones among children in “Talab Sarai” Punjab Pakistan

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Abstract: 190 children aged 7-18 years from an endemic fluorotic village “Talab Sarai” ($n = 130$) and a non-fluorotic, control, village “Ottawa” ($n = 60$) were selected for comparison. Children were examined for fluoride (F^-) concentration in drinking water, urine, and serum as well as Dental fluorosis (DF) and thyroid hormone levels. The mean concentration of water fluoride (WF) in the sample group was 6.23 mg/L, urine fluoride (UF) 3.38 mg/L, and serum fluoride (SF) 0.21 mg/L, while DF was 93.07%. Significant elevations ($P = 0.000$) in the concentration of all these four variables were observed in sample group children as compared to control. Mean Free Tetra-iodothyronine (FT4), Free Tri-iodothyronine (FT3) and Thyroid Stimulating Hormone (TSH) concentrations in the sample group were 16.64 pmol/L, 5.57 pmol/L and 4.41 mIU/L, respectively. No marked difference in FT4 ($P = 0.1$) was noted, while significant elevations in FT3 and TSH ($P = 0.000$) were found in the sample relative to the control group. 80% of the children displayed clear thyroid hormonal derangements, with 36.92% having high TSH and 43.07% with FT3 and FT4 disorders. A moderate to strong correlation among WF, UF, SF and DF ($r = 0.94, 0.60, 0.60, 0.72$) and a very strong correlation between WF and TSH ($r = 0.9$) were observed. Our results suggest that excess F level that is four times greater than the “safe limit” is not only increasing fluoride concentration in

body fluids but is also affecting thyroid hormones in 4 out of 5 children which could lead to abnormal physical and mental growth in later developmental stages.

Keywords: Water fluoride; Urine and Serum fluorides; Dental fluorosis; FT4; FT3 TSH.

1 Introduction

Fluoride (F^-) is micronutrients that is widely disseminated in the biosphere. Once it is ingested through the gastro intestinal tract, calcified tissues quickly absorb it. A very serious health complication associated with high F-consumption is fluorosis, which involves mottled tooth enamel, also known as dental fluorosis (DF), in early stages and bone deformity (skeletal fluorosis) later on [1]. DF is most prevalent in regions of the world where F-concentrations in household drinking water are greater than 1 mg/L.

Ground water with elevated F- levels occur in various parts of the world. As reported by the World Health Organization (WHO) in 2006, there are more than 29 countries (both developed and developing) which are endemic for fluorosis across the world [2]. Fluorosis prevalence of approximately 32% has been documented in various regions of the world [3].

F- concentrations of less than 1 ppm are beneficial for decreasing dental caries, but greater than 2 ppm result in accumulation of fluoride in the body, affecting teeth, bones and, most importantly, thyroid hormone production [1].

Thyroid hormones regulate body temperature, appetite, energy levels, growth, skeletal development, muscle tone and agility, cardiac rate, fluid balance, blood sugar levels, central nervous system functions, bowel functions, blood fat, carbohydrate and protein metabolism, and many other factors. Research has shown that the thyroid gland is the body organ that is most sensitive to F- [4]. Desun

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elaborated the abundant effects of F⁻ on the synthesis of thyroid hormones [5], and. Yaming reported that the long run consumption of F⁻ is a risk factor for the development of thyroid problems [6]. Lin and Wu demonstrated an elevation of TSH in mice, rats and pigs due to excessive F⁻ intake [7,8]. Most significantly, studies have shown that interference of F⁻ with thyroid hormone metabolism is a potential source of decline in brain activities and abnormal development in children [9].

Approximately sixty types of industries are contaminating air and water with excess F⁻, for instance, chemical production units, waste puddles, bricks manufacturing, and aluminum fertilizers as well as , steel, glass, tile, enamel, pottery, cement, metal casting, welding and brazing and magnesium foundries [10,11]. However, the major source of F⁻ consumption is drinking water: foods prepared with F⁻ contaminated water can make a significant addition to F⁻ ingestion, especially in teenagers [12].

Dietary intake has currently been established as a path which can produce DF [13], which is an extremely common disorder. It is, characterized by hypo mineralization of tooth enamel caused by ingestion of excess F⁻ during enamel formation. In moderate to severe fluorosis, teeth are physically damaged.

In addition to food, even salt and other, common spices and food dressings can be sources of F⁻ accumulation in body. The concentration of F⁻ content in tea is quite high among drinks and showed a range from 122-260 ppm in various brands with a mean of 186 ppm [14]. F⁻ containing toothpaste is another important source of excess F⁻ intake. Usually up to 20% toothpaste is absorbed per brushing, and on an empty stomach 100% F⁻ gets absorbed.

Ground water with elevated F⁻ level (> 1.5 ppm) is commonly observed in India, China, Sri Lanka, Spain, Italy, and the West Indies. One of the most well-known high F⁻ belts extends along the East African Rift from Eritrea to Malawi. A second belt passes through Iraq, Iran, Afghanistan, Pakistan, India, Northern Thailand and China. Identical belts exist in the Americas and Japan, and represents a potential source of increased F⁻ in drinking water [2].

“Non-ionic fluoride” is the main form in which F⁻ exist in human serum. Many studies have depicted a positive correlation between water and serum fluorides and their influence on thyroid hormones [13].

Some, very limited, studies from ‘Punjab,’ particularly from the its rural areas, are available regarding excess F⁻ in water and body fluids [16,17,18], but their effect on thyroid hormones has not yet been studied. In order to prevent F⁻ toxicity, it is necessary to analyze the ground water of fluorosis- affected regions and to study its consequences

on the natives, particularly children in different stages of puberty, as most of the growth and development takes place during this period.

One such village, “Talab Sarai,” is examined in present study. It is located 4.95 km southwest of “Manga Mandi,” a known endemically fluorotic territory. This village was chosen due to very high F⁻ in ground water, excessive teeth browning, and common symptoms of skeletal fluorosis in children. In this area, up till now, no such work has been available to shed light on concentrations of F⁻ in household drinking water including: its levels in human urine and serum, especially in children, and its relation to dental deformities and thyroid aberrations, if any. Therefore, the purpose of this study was to determine the concentration of F⁻ in ground water, urine and serum of the studied subjects and the magnitude of DF and level of thyroid hormones along with their derangements, and then to compare and correlate these parameters with a control group of the same age and socioeconomic conditions from a non-fluorosis territory .

2 Experimental

2.1 Subject Selection

Two villages about 90.4 km apart, namely Talab Sarai (TbS) and Ottawa (OTW), were selected for examination. TbS is situated in an endemically fluorotic territory with prominence of excess F⁻, mottled enamel and joint stiffness in the affected population. Ottawa, approximately 65 km from Lahore, was selected for control because it is a non-fluorosis, non-endemic village. A total of 190 children aged 7-18 years were chosen randomly from both villages after initial water testing. Written and informed consent of participation was obtained from the guardians/parents of every child. Those who did not agree, who were suffering from any health problem, or were not residents by birth were excluded from the study. The clinical protocol for this work was approved by the ethical committee of Institute of Chemistry, University of the Punjab. Along with collection of water, urine and serum from children, teeth of every child were examined by a dentist for the estimation of mottled enamel.

2.1.1 Reagents and Instruments

The following chemicals and instruments were used during the course of study:

Fluoride Ion Selective (combination) Electrode (Hanna HI 4110), pH/ISE/mV Meter (Hanna HI 4222), Electrode Holder (Hanna HI 76404), Magnetic Stirrer (Hanna HI 180), Plastic Beakers (Hanna HI 740036 P), Gamma Counter (Cap-RIA 16 CAPINTEC; Inc. USA), Vortex type Mixer (Barnstead Internationals-M 63210-33), Shaker (Gallenkamp- OSM-747), 500 UL Semi Programmed Pipette (Eppendorf), Small Test Tubes (Specially designed 5cm long, ϕ 14 mm, Fluoride Standard Solution 1000 ppm (Hanna HI 4010-03), Total Ionic Strength Adjustment Buffer (TISAB II, Hanna HI 4010-00), Reference Fill Solution (Hanna HI 7075), Deionized Water ($<18\text{M}\Omega/\text{cm}$) for dilutions, FT4, FT3, TSH kits by Immunotech Inc. (Beckman).

2.1.2 Method used for Fluoride Analysis

F⁻ measurements in water and body fluids were carried out with an F⁻ ion selective (combination) electrode. The use of an ion selective electrode (ISE) is not very common in Pakistan, but this methodology was preferred because of its lower expense relative to other analytical procedures, such as Atomic Absorption Spectroscopy or Ion Chromatography. This method is simple and robust, and provides frequent measurements. It has wide range of applications and can be used over a broad concentration range. It provides accurate measurements in the range of 0.01 – 127,000 ppm.

2.2 Sampling and Testing

2.2.1 Water Collection

Collection of water samples was done in three successive visits from May-August 2017. A sample of drinking water was collected from each child's house, typically from the hand tap, which involves a (hand pump or electric motor reaching tube wells of the Water and Sanitation Agency (WASA). Sampling was done in prewashed, pre-cleaned, pre-coded polyethylene bottles. At sampling sites, each bottle was rinsed thrice with sampling water, and the tap was kept open for three minutes to get a representative sample. Plastic gloves were used to prevent any hand contamination in the sample. After collection each sample's color, odour, and taste was recorded by physical examination while pH was determined immediately with a pH meter (Hanna HI 4222). All samples were moved to the laboratory on the same day and frozen at -20°C . They were thawed and analyzed for F⁻ content within a week.

2.2.2 Urine Collection

Clean and coded polyethylene containers containing 0.2 g EDTA were provided to each participant, and casual urine samples were obtained "on the spot." Mothers of children aged less than 12 helped their children, while older children provide urine samples themselves. Each urine sample, after packing in a separate polyethylene zipper, was placed in an ice box and moved to the laboratory and analyzed for F⁻ content on the collection day.

2.2.3 Blood Serum Collection

With the help of new sterilized syringes, 5-7 ml blood was drawn intravenously from each participant. For clotting purpose, blood was immediately transferred into open glass vials for about 30 minutes. Then they were centrifuged at 4000 rpm for 10 minutes, which resulted in the separation of serum from blood cells. Serum was transferred to another vial and capped. Serum vials were kept frozen at -20°C until analysis [19].

2.2.4 Dental Fluorosis Survey

Due to the very common occurrence of teeth browning at TbS, an experienced dentist examined the teeth of all participating children in both of the villages. Teeth analysis was carried out with the help of mouth mirror, forceps and probe under ordinary light. Dean's fluorosis index (revised six-point scale (1942)) was used to evaluate the grade of DF [20] according to the following scale.

Dean's fluorosis index						
Levels	0	1	2	3	4	5
Description	Normal	Questionable	Very mild	Mild	Moderate	Severe

2.3 Statistical Analysis

In order to compare the means of water, urine, serum fluorides, DF, FT4, FT3 and TSH among sample and control groups, student t-test using unequal variances was applied. 95% confidence interval (CI) and *P*-value less than 0.05 was assumed to indicate statistical significance. The Kolmogorov-Smirnov test was used in order to determine the normality distribution of the data wherever required. For finding correlations among WF, UF, SF, DF, FT4, FT3 and TSH, 'Pearson correlations' and "regression analyses" were used at same CI and *P*-

value. For comparing different groups of D.F with FT4, FT3 and TSH, means, standard deviations, student t-tests and ANOVA (single factor) were used. In order to find the variation between male/female and low, normal and high concentrations of FT4, FT3 and TSH, Chi square tests were employed.

2.4 Sample Preparation for Fluoride Analysis

A F- (ISE) (combination type (HI-4110) was used for the determination of F- in water, urine and serum. Deionized water with resistance less than 18 Ω /cm was used for dilution. The F- ISE was first calibrated with different F- standards and then analysis of individual samples was performed. The electrode was recalibrated with at least three F- standards after 40 minutes of use to ensure accurate measurements. Between samples, the electrode was washed with deionized water and dried before the next use.

2.4.1 Water Samples

To one part of sample, one part of total ionic strength adjustment buffer (TISAB II) was added. F- standards for the analysis were kept in the range of 0.1- 10 mg/L [16]. WF outcomes were presented as less than 10% Coefficient of variation (C.V) of the impression profile. To evaluate accuracy and validity of applied methods, water samples were spiked with F- standard dilutions. Recoveries were measured in the 0.2-3.2g range, and they resulted in spreads of 96.7-99.8% (mean 99%).

2.4.2 Urine Samples

For UF estimation, the same ratio of urine sample and TISAB II was used. A 0.5-5.0 mg/L range of F- standards was maintained to cover the wide range of F- concentration in the samples. Outcomes of the determination were presented as 10% C.V of impression profile. To measure accuracy and validation of the method, urine samples were spiked with F- standards. Mean percentage recovery was 99.1% in a range of 98-101.3%.

2.4.3 Serum Samples

Blood serum and TISAB II were combined in 1:1 ratios for estimation of F- in serum. For calibration purpose, F-

standards were prepared in the range of 0.01-1 mg/L. In inter and intra assay precision, the means and C.V were 0.23% and 0.22%, respectively. Mean percentage recovery was observed to be 98%, with a range of 96-101.4%, which supports the accuracy and validity of the applied method.

2.5 Free T4, T3 and TSH Determination

Competitive Radioimmunoassays (RIA) and sandwich-type Immunoradiometric Assays (IRMA) were used to estimate Free T4, T3 and TSH levels by using commercial kits of Immunotech Inc. (Beckman, Czech Republic). A computerized Gamma counter (Cap- RIA 16,CAPINTEC; Inc.USA) was used for the computation of radioactivity, adjustment of standard curves and sample analysis. Commercially- obtained control sera of at least three different concentrations were incorporated in each run to estimate the assay reliability. A maximum 10% C.V of the impression profile was used to express the results of RIA and IRMA.

3 Results and Discussion

One hundred and ninety children aged 7-18 years with average age of 12.40 ± 3.3 years were examined during the study. The sample group included 130 children (80 male and 50 female) from an endemic fluorosis village, Talab Sarai (TbS), which is near Manga Mandi. The control group was selected from a non-fluorosis village: Ottawa (OTW), 65 km from Lahore. The control group consisted of 60 children (30 male and 30 female) with an average age of 12.13 ± 3.2 years.

At TbS, 84.6% water samples had F- concentrations greater than 1.5 mg/L (WHO permissible limit). In the control group, WF ranged between 0.13 and 1.1 mg/L.

The results (Table 1) indicated that the concentration of F- in the drinking water of the sample population was significantly ($P = 0.000$) elevated relative to that of the control group. The mean concentration of UF in sample and control villages is also presented in Table 1. Levels of urinary fluoride in TbS was clearly higher than that of the control ($P = 0.000$). 90.5% of urine samples from the sample village had F- concentrations more than the normal upper limit of 1 mg/L as defined by Jaganmohan [21].

A scatter plot of WF vs. UF from the sample village is shown in Figure 1. The Pearson correlation coefficient ($r = 0.94$) suggested a very strong correlation between the two; in other words, these data are consistent with the idea that an excess amount of F- in water is a major cause of F- elevation in urine of children.

Table1: Level of Fluoride in Water, Urine and Serum.

Village s	Number (No.) of samples	Water Fluoride (mg/L)	Urine Fluoride (mg/L)	Serum Fluoride (mg/L)
TbS	130	6.23 ± 3.31^a 0.1-14.1 ^b	3.38 ± 1.47 0.1 -7.5	0.21 ± 0.1 0.01 – 1
CNTL	60	0.54 ± 0.15 0.13 -1.1	1.52 ± 0.43 0.52 - 2.3	0.05 ± 0.04 0.017- 0.45

a = Mean $\pm$ Standard Deviation

b = Range

Note: Water Fluoride $P = 0.000$ (student t-test using unequal variances)Urine Fluoride $P = 0.000$ Serum Fluoride $P = 0.000$ **Figure 1:** Scatter chart between Water and Urine Fluoride of sample Group Pearson Correlation Coefficient $r = 0.94$.

The mean SF in the sampled subjects was 0.21 ± 0.12 , and 72.3% ($n = 94/130$) of subjects were found to have SF levels higher than the standard Limit [22]. These results are further strengthened by the clear difference in the SF concentrations of sample and control groups ($P = 0.000$, Table 1). A scatter chart of WF vs. SF exhibited a positive correlation ($r = 0.56$). A similar outcome was observed in the relationship between UF and SF ($r = 0.60$) of the sample group. These results suggest that elevated F- in drinking water is a main cause of excess F- excretion by the human body.

Concentrations of FT4 in children of “TbS and OTW” are presented in Table 2. Student t-test (unequal variances) showed no statistically significant difference ($P = 0.10$) in the mean concentration of FT4 between the two groups. Mean FT3 and TSH levels of sample versus control group showed clear differences, ($P = 0.000$, Table 2).

The relationships of FT4 and, FT3 in serum with drinking WF among sample and control subjects displayed

no statistically significant positive correlations; however, a strong positive relationship is seen between TSH and WF ($r = 0.90$, Figure 2).

To evaluate the levels of DF, all 190 participants were examined. 93.07% from TbS and 7% from OTW presented with this issue. The changes observed in tooth surfaces include white flecks, coverage of one quarter to one half of tooth surfaces with opaque paper white areas, marked wear on biting surfaces, brown stains, convergent pits, and complete tooth surface damage.

Comparison of various degrees of DF (0-5) with their respective mean FT4, FT3 and TSH is presented in Table 3. Levels of mean FT4 varied considerably in DF levels 1-4, and mean TSH levels differed throughout the range of the fluorosis index, excepting level 3. FT3, on the other hand, displayed no apparent relationship to D.F.

Comparison of mild, moderate and severe DF with FT4, FT3 and TSH by an “ANOVA single factor” test suggests a detectable difference between FT4 and the

Table 2: FT4, FT3 and TSH Concentration in Serum of Subjects of the two Villages.

Villages	No. of samples	Mean FT4 (pmol/L)	Mean FT3 (pmol/L)	Mean TSH (mIU/L)
TbS	130	16.64 ± 3.78 8.5-33.5	5.57 ± 1.01 3.34 -9.7	4.41± 2.29 0.98-13.2
CNTL	60	17.55 ± 3.43 11.02-23.08	5.09 ± 0.53 2.81-6.0	2.36 ± 1.06 0.54- 6.12

a = Mean ± Standard Deviation

b = Range

Note: FT4: $P = 0.100$ (student t-test using unequal variances)FT3: $P = 0.000$ TSH: $P = 0.000$ **Figure 2:** Scatter chart between WF and TSH of sample Group $r = 0.90$.

degree of DF but that this difference did not reach a statistically- significant level ($F = 2.44$, $P = 0.09$). The identical result is observed in the case of FT3 ($F = 0.85$, $P = 0.43$); however, in case of TSH, the difference was statistically significant ($P < 0.05$) (Figure 3). In TbS, severe (score 5) DF was observed most frequently (Figure 4), indicating a prominent influence of endemically fluorotic territory on tooth development. WF also presented a positive dose relation ($r = 0.72$) with DF, confirming the powerful influence of F- intake on tooth development. High, normal and low concentrations of FT4, FT3 and TSH by gender and age groups in TbS and control were also analyzed. The normal laboratory ranges of FT4, FT3 and TSH are 11.5-23 pmol/L, 2.5-5.8 pmol/L and 0.3-5 m IU/L, respectively [23]. Our results showed that 5,1 males (5 from TbS and 1 from control) and 2,1 females had elevated FT4, 23,1 males and 16,1 females had elevated serum FT3 while 24,1 males and 19,1 females had high serum TSH in TbS and controls group, respectively.

Table 3: Comparison of Different Grades of D.F with FT4, FT3 and TSH in Sample Group.

D.F Grades	FT4 (P-value)	FT3 (P-value)	TSH (P-value)
1- 2	0.40	0.72	0.00
1-3	0.04	0.23	0.04
1-4	0.74	0.21	0.01
1-5	0.38	0.34	0.00
2-3	0.04	0.38	0.04
2-4	0.05	0.34	0.22
2-5	0.00	0.57	0.00
3-4	0.05	-----	0.01
3-5	0.01	0.56	0.00
4-5	0.34	0.45	0.00

Figure 3: Comparison of Thyroid Hormones in Mild, Moderate and Severe D.F at TbS.

Figure 4: Percentage Distribution of Dental Fluorosis among Sample Group.

Both groups showed significant differences with respect to the number of children having elevated thyroid hormones ($P = 0.000$). The use of a Chi-square test indicated no statistically significant difference between males/females and level of FT4, FT3 and TSH in sample and control villages.

Concentrations of F- greater than 1.5 mg/L in drinking water have been shown to result in various kinds of fluorosis, renal and neurological abnormalities and myopathy [24,25]. In the present study, geological causes, fertilizers used in agriculture, and industrialization, particularly brick kiln units (dozens are in use in the surrounding area), are the major likely contributors of F- in ground water [26]. TbS lies on a well known F- belt, which is: rich in F- bearing minerals like fluorspar, cryolite

fluorapatite and hydroxyapatite [27,28]. The weathering of these rocks and infiltration of rain through them increases the concentration of F- in ground water. All these sources added up to increase the level of F- in ground water (mean 6.23 mg/L at TbS as compared to 0.54 mg/L at the control site (OTW). Farooqi et al. (2007) found WF in the range of 2.47-21.1 mg/L in a nearby village, "Kalalanwala," while Ahmed (2001) observed 86% water samples with fluoride concentrations greater than WHO limits (1.5 mg/L) in the same village [29,30].

This territory has a semiarid and subtropical continental climate characterized by sultry summer and cool winters. The mean temperature in summer in Punjab is 41°C [29]; in such climatic conditions, F- level in drinking water should not be more than 0.7 mg/L [31] because, the

average water intake per child from April-August is 2.5 L, and 86-97% of F- from water directly becomes the part of body [32,33].

Fluoride toxication is most accurately computed in the form of urine and serum fluorides [34]. Normal limits of F- in urine and serum is 1.0 and 0.15 mg/L, respectively [21]. Kidneys are the main excretory organs for removal of absorbed fluoride via urine [6]. Chronic kidney problems are often seen in early aging society, and these problems lead to gradual declines of kidney function that may lead to permanent kidney failure, or even end-stage renal disease [35]. 3.38 mg/L (mean UF) and 0.21 mg/L (mean SF) in teenagers at TbS in this scenario is alarming.

The fact that very high fluoride concentrations in human body are induced from drinking water is strengthened by the positive correlations between WF-UF ($r = 0.94$),

WF-SF ($r = 0.60$) and UF- SF ($r = 0.60$). Similar findings are also reported by Qayyum et al. in "Sham Ki Bhatian," a nearby village in Punjab, Pakistan [16,17].

About 50% of the F- intake becomes part of the calcified tissues within 24 hours [36]. This absorption is very active in adolescence and decreases with increases in age [8]. The present investigation displayed an increment in DF with an increase in household WF ($r = 0.72$). Viswanathan in South India also reported similar outcomes [8] under identical conditions. 93.07% mottled enamel was found in sampled children from TbS. It is not only a visible decoloration of tooth enamel but also a developmental disorder which indicates aberrant thyroid hormone metabolism. While comparing the serum free T4, T3 and TSH levels of sample group to those of controls, no significant alteration in FT4 level ($P = 0.10$) was seen but serum FT3 ($P = 0.000$) and TSH ($P = 0.000$) revealed clear differences between the two groups,

supporting the influence of SF on thyroid hormones. In the present investigation, weak to moderate, but statistically significant, correlations were found between SF-FT4, SF-FT3 and SF-TSH ($r = 0.1$, $P = 0.000$, $r = 0.14$, $P =$ and $r = 0.60$, $P = 0.000$, respectively). These correlations support the role of SF in altering the level of thyroid hormones in the human body. In 2008, Wu observed analogous serum- thyroid correlations in animals [36].

In the present study, the effect of drinking water in elevating the thyroid hormones is noticed in TSH ($r = 0.9$, $P = 0.005$) but not in FT4 or FT3 : WF- FT4 ($r = 0.1$, $P = 0.000$) and WF- FT3 ($r = 0.03$, $P = 0.000$). Similar increases in TSH levels were observed by AK Shusheela in children living in an endemic fluorosis area in India [37]. According to the National Research Council (NRC), TSH is considered a "precise and specific barometer" of thyroid status". Fluoride could take part the subclinical hypothyroidism,

which is a condition of "mild thyroid failure" marked by increased TSH and normal T3/T4.

Further compilation of results from this endemically fluorosis village indicated 79.23% well defined thyroid hormonal derangements in children: 36.92%, 30.77%, 5.38% had elevated TSH, FT3 and FT4 whereas 6.15% depicted low FT4 which give rise to following thyroid disorders:

- 23.85% ($n = 31$) had high TSH with normal FT3-FT4, which is: the first sign of thyroid malfunctioning known as "subclinical hypothyroidism". Mild subclinical hypothyroidism in mothers can give rise to lower IQ and hearing impairment in children. As hearing development is under control of thyroid hormones, thyroid malfunctioning in late fetal and initial postnatal life severely affect the acoustic organ, as indicated from the deafness linked with congenital hypothyroidism. [38]
- 9.23% ($n = 12$) presented with high TSH-FT3 but normal FT4 which are the initial symptoms of "T3 Toxicosis". This problem is associated with the elevation of T3 levels due to the disruption in deiodination of T4 to T3. The problem manifests in several ways, including anxiety, fatigue and weight loss [39].
- 3.85% ($n = 5$) presented with elevated FT4 but, normal FT3-TSH. These are the first signs of "Hyperthyroidism," which gives rise to an elevated metabolic rate, which is: characterized by rapid heart rate, high blood pressure, weight loss, nausea, vomiting, and other signs.
- 2.31% ($n = 3$) had low FT4, high TSH and normal FT3. These levels are key indicators of primary hypothyroidism and iodine deficiency disorder, result in lethargy, depression and weight gain later on.

Identical hormonal abnormalities are also noticed in children of Dehli, India and as reported by the NRC (review of literature 2006) [37]. Such a large group of children with abnormal FT4, FT3 and TSH levels relative to controls groups ($P = 0.000$) suggests an important effect of high F- intake on thyroid function. Similar results are also expressed by Xiang, Susheela, and Singh regarding other regions of the world [41,37,41].

When comparing different degrees of DF (from normal to severe) with their respective mean FT4, FT3, TSH values, we noticed a significant alteration in FT4 concentrations in questionable- mild, very mild-mild, very mild- moderate, very mild- severe, mild-moderate and mild-severe grades on the fluorosis index (Table 3). No statistically significant difference in DF grades and FT3 concentration were obtained. DF and TSH revealed considerable differences

in all grades except very mild-moderate levels (Table 3). Identical outcomes can be seen in F- and thyroid functions in two villages of China [42].

Comparison of mild, moderate and severe DF with their respective thyroid hormones present significant difference in mean TSH level. Identical outcomes were also noted by MB Hosur [43] in teenagers. These facts are advocating the role of mottled enamel as “developmental disorder that” is related to disturbance of thyroid activities.

4 Conclusion

It is obvious from the outcomes that the concentration of F- in drinking water of “Talab Sarai” is dangerously high, which is elevating its level in urine and serum of the resident adolescents. Increased F- in body fluids affects the production mechanism of thyroid hormones as this endocrine gland is the most F- sensitive organ in the human body. Results suggest that 93.07% teeth fluorosis and 80% thyroid hormone abnormalities in the sample area confirm excessive deterioration due to F- intoxication. Positive dose relationships between F- in water with all studied parameters (urine, serum, DF, thyroid hormones) strengthens our understanding of the immense role of F- towards abnormal growth, especially bones and teeth in children. There is a serious need to take preventive measures such as water purification and, use of calcium and, vitamin C, D and E supplements along with additional mapping of this endemic fluorosis territory.

Conflict of interest: Authors declare no conflict of interest.

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