Original Article

Fluoride and Thyroid Function in Children Resident of Naturally Fluoridated Areas Consuming Different Levels of Fluoride in Drinking Water: An Observational Study

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Abstract

Background: Literature shows association between systemic fluorides and endocrine disorders especially related to thyroid, with lack of clarity. **Aims and Objectives:** The aim and objective of this study was to estimate serum triiodothyronine (T3), thyroxin, thyroid-stimulating hormone (TSH), fluoride, calcium, phosphate, and alkaline phosphatase levels among children with normal nutritional status and optimal iodine intake residing in three different ranges of drinking water fluoride levels. **Materials and Methods:** The present double-blinded, observational trial comprised of 293 children aged between 9 and 13 years consuming naturally fluoridated water of three different ranges: Group I: 0.01–0.6 parts per million (ppm), Group II: 0.7–1.2 ppm, and Group III: 1.3–1.8 ppm. For each child's demographic data, body mass index and Clinical Fluorosis Index were recorded along with serum T3, T4, TSH, fluoride, calcium, phosphate, and serum alkaline phosphatase levels. Data were analyzed using Chi–square test, Kruskal–Wallis test, and repeated measures ANOVA with SPSS 23. **Results:** For serum TSH levels, 40% of children in Group I had deranged levels followed by Group III (20%) and Group II (16%). For serum T4 levels, 24% of children of both Groups I and III had deranged levels followed by Group II (20%). Intergroup correlation of drinking water fluoride levels to the number of deranged serum T3, T4, and TSH of the children showed nonsignificant association. Serum T3, calcium, phosphate, and alkaline phosphatase levels in all children showed values falling within normal range. **Conclusion:** According to the present study results, long-term intake of fluoridated drinking water (0.02–1.4 ppm) did not show effect on the thyroid function in children with normal nutritional status and optimal iodine intake.

Keywords: *Iodine, nutrition, serum fluoride, systemic fluoride, thyroid function test*

Introduction

Fluoridation of community water supplies is the defined adjustment of fluoride levels in drinking water to an optimal level for the prevention of dental decay. Studies conducted in the past 60 years across the world have consistently indicated that fluoridation of community water supplies is effective in preventing dental decay in both children and adults. The Centers for Disease Control and Prevention stated that community water fluoridation is one of the ten great public health achievements of the 20th century.[1-3] Despite its merits, community water fluoridation is a controversial public health intervention. Allegations include increased overall mortality and occurrence of Down syndrome,[2] specific cancers, endocrine disorders, and behavioral, cognitive, and other neurological ill effects.[2,3]

Thyroid diseases are one of the commonest endocrine disorders worldwide including India, with about 42 million people suffering from it.[4] Considering a potential association between fluoride exposure and endocrine disruption especially thyroid, many *in vitro* experimental, animal, and human studies have been published with more concern on thyroid.[5-15] However, there exists a lack of clarity. Few studies reported that excessive long-term intake of fluoride is a significant risk factor for the development of thyroid dysfunction.[7-9] One study in 1999 reported significant reduction in serum thyroxin (T4) with increased levels of triiodothyronine (T3) and thyroid-stimulating hormone (TSH).[13] Another study in 2001 reported

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T3 and T4 concentrations in the serum of patients with endemic fluorosis which were significantly below the normal reference value.[14] In contradiction, few studies have indicated that the high fluoride intake does not have any effect on thyroid function.[10-12]

The British Fluoridation Society (2006) based on the Royal College of Physicians, York Systematic committee, and World Health Organization International Programme on Chemical Safety review stated that "there is no evidence that fluoride is responsible for any disorder of the thyroid when consumed in optimal level."[15] The "optimal level" has been under revision through various authentic bodies over the years. Recently, the Department of Health and Human Services issued proposed recommendations to change the recommended optimal amount of fluoride in drinking water from 0.7 to 1.2 mg/L based on ambient air temperature to a uniform amount of 0.7 mg/L. Introduction of different fluoride delivery sources resulted in an uncoordinated delivery of fluoride in optimal doses, especially in naturally fluoridated areas.[16,17]

The present study was carried out with the objective to estimate serum T3, T4, TSH, calcium, phosphate, fluoride, and alkaline phosphatase levels among children with normal nutritional status and optimal iodine intake residing in three different ranges of drinking water fluoride levels. The research hypothesis was that long-term consumption of fluoridated drinking water at optimum or little above the optimal level affects thyroid function in children with normal nutritional status and optimum iodine intake.

Materials and Methods

Study design and ethical clearance

The study design was a double-blinded observational trial. The study plan was approved by the Institutional Ethical Committee. All information about the children and their identity was anonymous. After giving written information about the nature of the study, permission for selecting the samples was obtained from the concerned government and school authorities. Participants and their parents were explained about the nature of the study and written informed consent was obtained. They were informed to withdraw from the study at any point of time during the observational trial.

Study area selection

The children for the present study were selected from the villages of Mysore Taluk with the necessary drinking water fluoride level. Based on drinking water fluoride level reports of 2011 (district-level water quality testing laboratory),[18] 212 naturally fluoridated villages from 35 Gram panchayaths of Mysore were segregated into three categories as follows: below optimal 0.01–0.6 parts per million (ppm), optimal 0.7–1.2 ppm, and slightly above optimal 1.3–1.8 ppm. From each category, 15 villages were randomly selected for drinking water fluoride analysis.

Collection and analysis of water samples

From each selected village, after discussion with local in-charge person for water supply, the major drinking water sources were identified. From the identified sources, 500 ml of water sample was collected for analysis as per the American Public Health Association (APHA) (1998) guidelines in sterile high-density polyethylene bottles.[19] The collected samples were labeled and transported to laboratory for further analyses. Water analysis was carried out in the research unit, according to analytical protocol using OAKTON Fluoride Ion Selective Electrode (ISE) Equipment, USA. Each sample was analyzed twice for confirmation. The analyzed drinking water fluoride concentration of each village was tabulated. Of the 45 villages analyzed for drinking water fluoride levels, 26 villages showing variation in fluoride levels from that of 2011 report were excluded because the present study focused on the long-term effects of fluoride. Of the selected 19 villages for the present study, 6 villages were with water fluoride level at 0.01–0.6 ppm, 8 villages were at 0.7–1.2 ppm, and 5 villages were at 1.3–1.8 ppm.

Subject selection

From the 19 identified villages, all 1036 schoolgoing children between the ages of 9 and 13 years were screened for selection criteria. Schoolchildren were targeted for high, expected levels of cooperation and low population mobility. Demographic information of the children was collected from the school records. Information regarding town of residence, source of water use from birth to age 3, and current water source for both cooking and drinking was collected by interviewing the parents. The adequacy of the children's nutritional status was accessed through the Indian Academy of Paediatrics (IAP) Growth Charts (Revised IAP Growth Charts for Height, Weight and Body Mass Index for 5 to 18-year-old Indian Children-2015)[20] for which anthropometric measurements of the children were collected by weighing the children on a calibrated load cell-operated electronic scale accurate to 50 g while they were dressed in light clothing without shoes and measuring body heights with a stature meter containing a metal tape accurate to 0.1 mm. A total of 293 healthy children with good general health, lifelong residents of their particular locality, normal nutritional status, consuming iodized salt, and who is willing to participate in the study and gave written informed consent were included in the study. The included children based on their drinking water fluoride levels were stratified into three groups: Group I: 0.01–0.6 ppm (98 children), Group II: 0.7–1.2 ppm (103 children), and Group III: 1.3–2 ppm (92 children).

Clinical examination and blood sample

A trained pediatric dentist carried out clinical examination to assess dental fluorosis. For each child, fasting venous blood samples (5 ml) from dorsal hand vein, as per protocol, were collected by trained and experienced

paramedical staff [Figure 1a]. The collected blood samples were transferred to pediatric vacutainer tubes, gently inverted, and labeled for patient's name, age, medical record number, and date [Figure 1b]. Collected blood was preserved in clean plastic centrifuge tubes and immediately centrifuged for 10 min at 6000 rpm. Serum was quickly removed to other clean plastic tubes and was kept in a refrigerator at −40°C until the analysis.

Analysis of blood sample

The level of serum T3 and T4 was determined with competitive chemiluminescent immunoassay (CLIA) kits, and the level of serum TSH was determined with ultra-sensitive sandwich CLIA with analyzer according to the manufacturer recommendation. Serum calcium levels were measured with ARSENAZO III system, serum phosphate levels were measured with ammonium molybdate system serum fluoride with ISE method, and serum alkaline phosphatase levels were measured with AMP buffer system. The collected data were tabulated and statistically analyzed. All the statistical operations were done through SPSS Version 23.0 statistical package (IBM corp., Washington DC, US). Data analysis was performed using paired *t*-test and repeated measures ANOVA.

Results

The mean age of the selected children fell within the range of 10.1–10.8 years with varied distribution of gender across the group. Drinking water fluoride level in Groups I, II, and III fell within the range of 0.12–0.32 (mean of 0.22.), 0.76–1.1 (mean of 0.83), and 1.43–1.46 (mean of 1.44), respectively [Table 1].

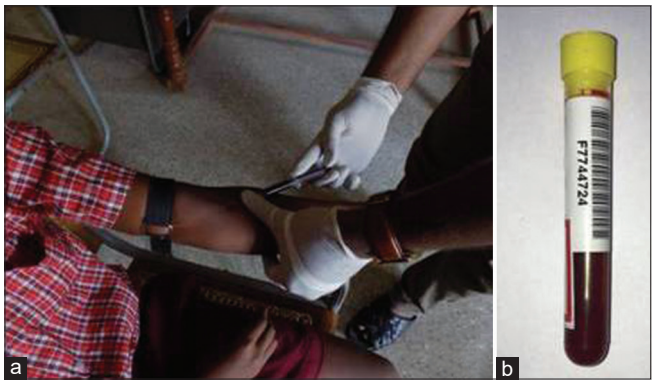

Figure 1: (a) Venipuncture. (b) Collected blood transferred to pediatric vacutainer tube

Mean serum fluoride levels of children across the groups showed an increasing trend from Groups I to III. Dental fluorosis evaluated through clinical Dean's fluorosis index across the groups showed maximum prevalence in Group III (88%) followed by Group II (85%) and least in Group I (4%) [Table 1].

Thyroid function

Among the 293 children evaluated for thyroid function consuming different levels of fluoride through drinking water, 66 (22.5%) children showed deranged levels.

For serum TSH levels, 40% of children of Group I had deranged levels followed by Group III (20%) and Group II (16%). Intergroup correlation for drinking water fluoride levels to number of deranged serum TSH for Group I to Group II, Group II to Group III, and Group II to Group I even though showed nonsignificant correlation, Group I showed more children with TSH derangement compared to other groups [Tables 2-4]. All the children with deranged TSH levels showed values falling between 4.5 and 10 mU/L, indicating children having subclinical hypothyroidism.

For serum T4 levels, 24% of children of both Groups I and III had deranged levels followed by Group II (20%). Intergroup correlation for drinking water fluoride levels to number of deranged T4 children by Chi-square test and Kruskal–Wallis test after comparison showed nonsignificant association [Tables 2-4]. Evaluation for serum T3 levels across the groups revealed that all the children had normal levels [Table 2].

Comparing mean TSH levels, Group I showed the highest value (3.86) followed by Group III (3.27) and Group II (3.21). Comparing mean T4 levels, Group I showed the highest value (9.53) followed by Group III (9.29) and Group II (8.79). Comparing mean T3 levels, Group III showed the highest value (158.36) followed by Group II (156.84) and Group I (140.80). Intergroup comparison for drinking water fluoride levels to mean serum TSH, T3, and T4 levels with ANOVA showed nonsignificant correlation [Table 5].

Serum calcium, phosphate, and alkaline phosphatase levels

Among the 293 children evaluated for serum calcium, phosphate, and alkaline phosphatase levels consuming

Table 1: Stratification of study participants according to gender across study groups with mean age, mean drinking water fluoride levels, mean serum fluoride level, and prevalence of dental fluorosis across the study groups

Group	Boys (%)	Girls (%)	Total (%)	Mean age (years)	Mean water fluoride level (ppm)	Mean serum fluoride level (ppm)	Prevalence of dental fluorosis* (%)
I (0.01-0.6 ppm)	39 (42)	59 (58)	98 (100)	10.1	0.22	0.03	4
II (0.7-1.2 ppm)	42 (41)	61 (59)	103 (100)	10.4	0.89	0.035	85
III (1.3-2.0 ppm)	55 (60)	37 (40)	92 (100)	10.8	1.44	0.05	88

*Deans Fluorosis Index; ppm: Parts per million

Table 2: Distribution of the study participants according to serum thyroid-stimulating hormone, triiodothyronine, and thyroxin levels across the study groups

Group	Number of cases with					
	Normal TSH (%)	Deranged TSH (%)	Normal T4 (%)	Deranged T4 (%)	Normal T3 (%)	Deranged T3 (%)
I (0.01-0.6 ppm)	77 (60)	21 (40)	74 (76)	24 (24)	98 (100)	0
II (0.7-1.2 ppm)	87 (84)	16 (16)	82 (80)	21 (20)	103 (100)	0
III (1.3-2.0 ppm)	74 (80)	18 (20)	70 (76)	22 (24)	92 (100)	0

TSH: Thyroid-stimulating hormone; T4: Thyroxin; T3: Triiodothyronine; ppm: Parts per million

Table 3: Serum thyroid-stimulating hormone and thyroxin - intergroup correlation

Group	TSH		T4	
	χ^2	*P*	χ^2	*P*
Group I-Group II	3.571	0.059	0.117	0.733
Group II-Group III	0.136	0.713	0.117	0.733
Group III-Group I	2.381	0.123	0.001	1.00

Pearson χ^2. TSH: Thyroid-stimulating hormone; T4: Thyroxin

Table 4: Serum thyroid-stimulating hormone and thyroxin levels - intergroup comparison

Thyroid function	Group	Mean rank	χ^2	*P*
TSH	I	43.50	4.312	0.116
	II	34.50		
	III	36.00		
T4	I	38.50	0.150	0.928
	II	37.00		
	III	38.50		

TSH: Thyroid-stimulating hormone; T4: Thyroxin

Table 5: Mean thyroid hormone levels across the study groups

Thyroid function	Group	Mean	SD	*P*
T3	Group I	140.8000	23.69072	0.280
	Group II	156.8400	28.91032	
	Group III	158.3600	22.13007	
T4	Group I	9.5360	1.69063	0.327
	Group II	8.7960	1.89043	
	Group III	9.2960	1.73048	
TSH	Group I	3.8680	1.77941	0.258
	Group II	3.2132	1.30446	
	Group III	3.2796	1.47656	

ANOVA. TSH: Thyroid-stimulating hormone; T4: Thyroxin; T3: Triiodothyronine; SD: Standard deviation

different levels of fluoride through drinking water, all children showed values falling within normal levels [Tables 6 and 7].

Discussion

Across the globe, approximately 25 countries are having artificial water fluoridation to varying levels and about 435 million people worldwide receive water fluoridation at the recommended level.[21] Twenty-eight countries have water that is naturally fluoridated, though in many of them the fluoride is above the recommended safe level.[21] India falls under naturally occurring fluoride region with more than twenty states endemic for dental fluorosis with 66 million people consuming naturally fluoridated water with varied concentrations. The recommended optimal fluoride level in drinking water is changed from 0.7 to 1.2 mg/L based on ambient air temperature to a uniform amount of 0.7 mg/L. However, according to the Bureau of Indian Standards 2009 Revision IS 10500, the permissible limit of fluoride in drinking water in the absence of alternate source is 1.5 ppm.[22]

Thyroid diseases are the most common endocrine disorders worldwide including India. In India, every seventh person is suffering from a thyroid disorder.[23] Literature reports a potential association between fluoride exposures and endocrine disruption, especially thyroid. Hence, it is of prime importance to address this issue.[7-9]

The present study was carried out with the objective to estimate serum thyroid function among children with normal nutritional status and optimal iodine intake residing in three different ranges of drinking water fluoride levels. It showed a nonsignificant association between thyroid function and long-term systemic intake of fluoride up to 1.4 ppm. The present study results show a nonsignificant association between dental fluorosis and thyroid function in children residing in near-optimal naturally fluoridated areas (0.2–1.4 ppm) with normal nutritional status and optimal iodine intake.

In accordance to our study results, Baum *et al*.[11] and Barberio *et al*.[24] reported that fluoride exposure does not increase susceptibility to impaired thyroid functioning at the population level. Our results are also in accordance with the endorsed statement quoted by the British Thyroid Association in 2006[25] and European Union's 2011 SCHER[26] report on health and environmental risks of fluoride that human studies do not suggest an association between water fluoridation and any thyroid disorder.

In contradiction to our results, studies by Wilson,[27] Murray *et al*.,[28] Steyn and Reinach,[29] Obel,[30] Jooste,[31] Rathee *et al*.,[32] Susheela *et al*.,[5] Xiang *et al*.,[33] Peckham *et al*.,[34] and Sachdeva *et al*.[35] found evidence of at least one or more thyroid hormone derangements among those deemed as having "high" fluoride exposure (defined in various ways). Lin *et al*.[36] found that

Table 6: Distribution of the study participants according to serum calcium, phosphate, and alkaline phosphatase levels across the study groups

Group	Number of cases with level, *n* (%)					
	Normal calcium	Deranged calcium	Normal phosphate	Deranged phosphate	Normal alkaline phosphatase	Deranged alkaline phosphatase
I	98 (100)	0	98 (100)	0	98 (100)	0
II	103 (100)	0	103 (100)	0	103 (100)	0
III	92 (100)	0	92 (100)	0	92 (100)	0

Table 7: Mean serum calcium, phosphate, and alkaline phosphatase levels across the study groups

Thyroid function	Group	Mean	SD
Calcium	Group I	9.244 mg/dl	1.04
	Group II	8.444 mg/dl	0.97
	Group III	8.974 mg/dl	1.35
Phosphate	Group I	4.044 mg/dl	1.63
	Group II	3.844 mg/dl	1. 43
	Group III	3.294 mg/dl	1. 04
Alkaline phosphatase	Group I	73.20 IU/L	1.12
	Group II	66.52 IU/L	16.37
	Group III	72.53 IU/L	9.56

Normal levels - Calcium: 7.8-11.4 mg/dl; Phosphate: 2.5-4.5 mg/dl; Alkaline phosphatase: 40-129 IU/L. SD: Standard deviation

individuals residing in high fluoride areas (defined as areas with an average fluoride concentration of 0.88 ppm in drinking water) had significantly higher TSH levels than those residing in low fluoride areas (average fluoride concentration of 0.34 ppm in drinking water) ($P < 0.01$). Michael *et al*.[37] found significant increases in T4 level in population with high fluoride exposure. Xiang *et al*.[33] reported that the high fluoride exposure can cause thyroid functional abnormalities. The reason for this contradiction may be the higher water fluoride levels of the study location (2.36–14 ppm) in comparison to ours (1.4 ppm). In addition, nutritional status and normal iodine consumption were not considered.

In the present study, normal nutritional status and optimal iodine intakes were considered as the selection criteria because studies reported greater possibility of endemic goiter in iodine-deficient communities, especially in low socioeconomic children with a poor nutritional status.[38] Dietary iodine intake has proved to influence the epidemiology of thyroid dysfunction. Iodine forms an essential component of the hormones produced by the thyroid gland.[39]

In areas where the daily iodine intake is <50 μg, goiter is reported to be endemic with a prevalence of 80% and when the daily intake is <25 μg, congenital hypothyroidism is observed.[40]

According to the World Health Organization, the simplest, most effective, and inexpensive preventive method is the consumption of iodized salt. In India, to combat iodine-deficiency disorders, it has been more than three decades since the Universal Salt Iodization program was introduced. India is undergoing a transition from iodine-deficient to iodine-sufficient state.[41]

A study by Jooste *et al.* in 1999[30] reported fluoride at 1.7 ppm and above may behave as a goitrogen in optimal iodine population. Our study results showed a nonsignificant association between thyroid function and fluoride up to 1.4 ppm.

Relating dental fluorosis to thyroid dysfunction, Schuld[42] reported that tooth and thyroid gland develop almost at the same age, so fluorosis and thyroid dysfunction may be correlated. Supporting the above hypothesis, a study by Xiang *et al.*[33] reported that the different severity degrees of dental fluorosis may be related to significant deviation in the serum levels of thyroid hormone. In contradiction, the present study results show a nonsignificant association between dental fluorosis and thyroid function. In accordance with our findings, a study by Hosur *et al*.[43] reported that children with dental fluorosis had normal thyroid hormone levels.

Mean serum fluoride levels of children across the groups showed an increasing trend from Groups I to III. Similar findings were noted in the study by Susheela *et al*.[5] and Singh *et al*.[44]

To carry out such a project, selection of study area plays a vital role. The need was an area with natural, independent, unaltered near-optimum ground water fluoride. Hence, we selected an area located at 12.30°N 74.65°E which has an average altitude of 770 m (2526 ft).[45] It is spread across an area of 128.42 km^2 (50 sq mi). It has a tropical savanna climate designated "Aw" under the Köppen Climate Classification with an average annual rainfall of 804.2 mm (31.7"). In the entire study area, groundwater is the major source for drinking water as centralized surface water supply is only restricted to the city limits.

In the present study, the final villages for sampling were selected based on drinking water fluoride levels estimated as per the APHA (1998)[19] guidelines using ISE method. Different methods and instruments have been used to estimate fluoride levels in drinking water such as ion chromatographic,[46] colorimetric,[47] potentiometric,[48] and spectrophotometric methods.[49] We preferred ISE method because of its advantages such as accuracy, speed, economical, and sensitivity in comparison with other methods such as ultraviolet-visible spectroscopy.

In the present study, the level of serum T3 and T4 was determined by competitive CLIA, and TSH was determined by ultra-sensitive sandwich chemiluminescent immuno assay. In the presence of complementary antigen and antibody, the paratope of the antibody binds to the epitope of the antigen to form an antigen-antibody complex or an immune complex. Estimating the levels of such immune complex by the use of labeled antibodies forms the basis of CLIA. It involves the use of stationary solid particles coated either with the antigen or antibody of interest for postincubation intact immune complex formation which generates light, the intensity of which is directly proportional to the amount of labeled complexes present which indirectly aids in the quantification of the analyte of interest. The intensity of light is measured in terms of relative light units.[50] The main advantage of this technology includes sensitivity and its ability to remain stable for background signals. Furthermore, the analyzers working under this principle are simple in design and operation.

Limitation

Dietary fluoride consumption strongly influences total fluoride intake. However, we had only considered drinking water fluoride level of children and this is one of the limitations of the present study.

Conclusion

Within the limitations of the present study, long-term intake of naturally fluoridated drinking water (within the range of 0.02–1.4 ppm) does not seem to have any effect on the thyroid function in the children with normal nutritional status and optimal iodine intake.

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Conflicts of interest

There are no conflicts of interest.

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