



# Relationship between water, urine and serum fluoride and fluorosis in school children of Jhajjar District, Haryana, India

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Received: 20 March 2016 / Accepted: 11 October 2016

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**Abstract** The present study was undertaken to determine the relationship between fluoride in water, urine and serum and dental fluorosis. The fluoride level in water and urine were measured spectrophotometrically by using acid zirconyl and SPADNS reagents, while the fluoride level in serum was determined by ion selective electrode meter. Dental fluorosis survey was conducted with the help of Performa prescribed by Rajiv Gandhi Drinking Water Mission and the use of Tooth Surface Index for Fluorosis. Mean fluoride values in water samples of Jhajjar City and Dadanpur and Dariyapur villages of Jhajjar District were measured to be 2.17 (range from 1.92 to 2.60 mg/L), 2.81 (range from 2.53 to 3.14 mg/L) and 2.22 mg/L (range from 1.63 to 3.33 mg/L), respectively. The mean fluoride values in the urine samples of children were found to be 1.51 (range from 0.05 to 2.64 mg/L), 1.71 (range from 0.69 to 2.80 mg/L) and 1.45 mg/L (range from 0.31 to 2.50 mg/L) at Jhajjar City and Dadanpur and Dariyapur sites, respectively. Serum fluoride was detected in the blood samples of children, who have high urinary fluoride at these three sites. The mean serum fluoride level was reported to be 0.15, 0.34 and 0.17 mg/L, respectively. A total of 842 children were also analyzed for dental fluorosis. The mean values of fluorosis-affected children in Jhajjar, Dadanpur and Dariyapur were 51.90, 94.63 and 36.84 %,

respectively. A significantly positive correlation between water, urine, serum fluoride concentration and fluorosis was seen.

**Keywords** Fluorosis · Jhajjar District · Serum fluoride · Urinary fluoride · Water fluoride

## Introduction

The problem of excessive fluoride in groundwater in India is common and was first reported by Short et al. (1937) from the state of Andhra Pradesh. At present, fluorosis is endemic in 21 states of India out of the 29, affecting more than 65 million people, including 6 million children (Jha et al. 2010; Verma et al. 2016; Rao et al. 2015). However, fluoride contamination of groundwater is a serious problem in several countries throughout the world (Kebede et al. 2016). Bhaumik and Mondal (2016) reported that the ingestion of excessive quantities of fluoride during pre-eruptive tooth formation results in chronic endemic fluorosis. Yadav and Lata (2002) have demonstrated that 10 % of the children were affected by fluorosis even when the concentration of fluoride in the water supply was 1.0 mg/L. Fluoride is excreted primarily via urine (IPCS 2002). Urinary fluoride is widely measured as an early indicator of fluoride poisoning among not only fluoride-exposed workers, but also inhabitants of fluoride-polluted areas (Zipkin et al. 1956). When the renal function deteriorates, the ability to excrete fluoride through kidney markedly decreases, possibly resulting in greater accumulation of fluoride in the body (Koichi et al. 1984; Ugran et al. 2016). Absorption and accumulation of fluoride in bones is influenced by a wide range of factors: endemic contamination, nutritional status, addition of fluoride to a public

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water supply, the consumption of bottled or carbonated water, age, and sex (Husdan et al. 1976). The estimation of fluoride in the plasma is a good index of fluoride status in the human system. Fluorides in human serum are found in two forms; ionic fluoride ( $F^-$ ) and nonionic fluoride (NF). The latter constitutes 80–90 % of total fluoride. The results of many studies suggest that serum  $F^-$  is directly correlated with water fluoride concentration (Li and Ke 1990; Ahemed et al. 2012; Arshad and Shanavas 2013).

Various studies from the state of Haryana have assessed the fluoride level in drinking water, urine and different food items (Yadav et al. 2006, 2007; Singh et al. 2007). Ingestion of excess fluoride, mainly through fluoride-contaminated drinking water, caused different types of fluorosis. It has become important to study the fluoride contents in the groundwater in the fluorosis-affected areas and assess its effect on human population, particularly children in rural areas, who are the most exposed to such contaminated water. In the present study, an attempt was made to understand the relationship between fluoride in groundwater, urine, serum and prevalence and severity of dental fluorosis.

## Materials and methods

### Study area description

The area of Jhajjar District is 1834 sq.km and geographically situated at 28°37'N latitude and 76°39'E longitude and 214 m above the mean sea level (Fig. 1). Jhajjar City, Dadanpur and Dariyapur villages were chosen for the present study because of the availability of water fluoride data and the high incidence of fluorosis among the locals. Residents of these sites use groundwater as the main source of drinking water. The groundwater sources include hand pumps, tube wells and open wells. The selected locations have more than 2.17 mg/L mean fluoride concentration, detected during a preliminary survey of the district (Yadav et al. 2008).

### Collection and fluoride determination in water samples

Random water samples from various drinking water sources (hand pumps, tube wells and open wells) were collected in an ice box from each location. A total of ten samples were collected from each selected location, representing the water quality of the whole area. The samples were transported to the laboratory and analyzed. Fluoride concentration was determined spectrophotometrically using acid zirconyl and SPADNS reagents (Bellack and

Schouboe 1968). Sodium fluoride was used to prepare the standard solution.

### Collection and fluoride determination in urine samples

To analyze the level of fluoride in urine, the spot urine samples of school-going children aged 8 and 15 years were collected. The urine composite samples of 24 h duration from 9 AM to 9 AM the next day were collected from each location. The urine samples were collected in non-reactive plastic containers and brought to the laboratory in an ice box and stored at 4 °C in a refrigerator. A total of 100 urine samples from each site were collected and analyzed for fluoride content using the SPADNS method.

### Collection and fluoride determination in serum samples

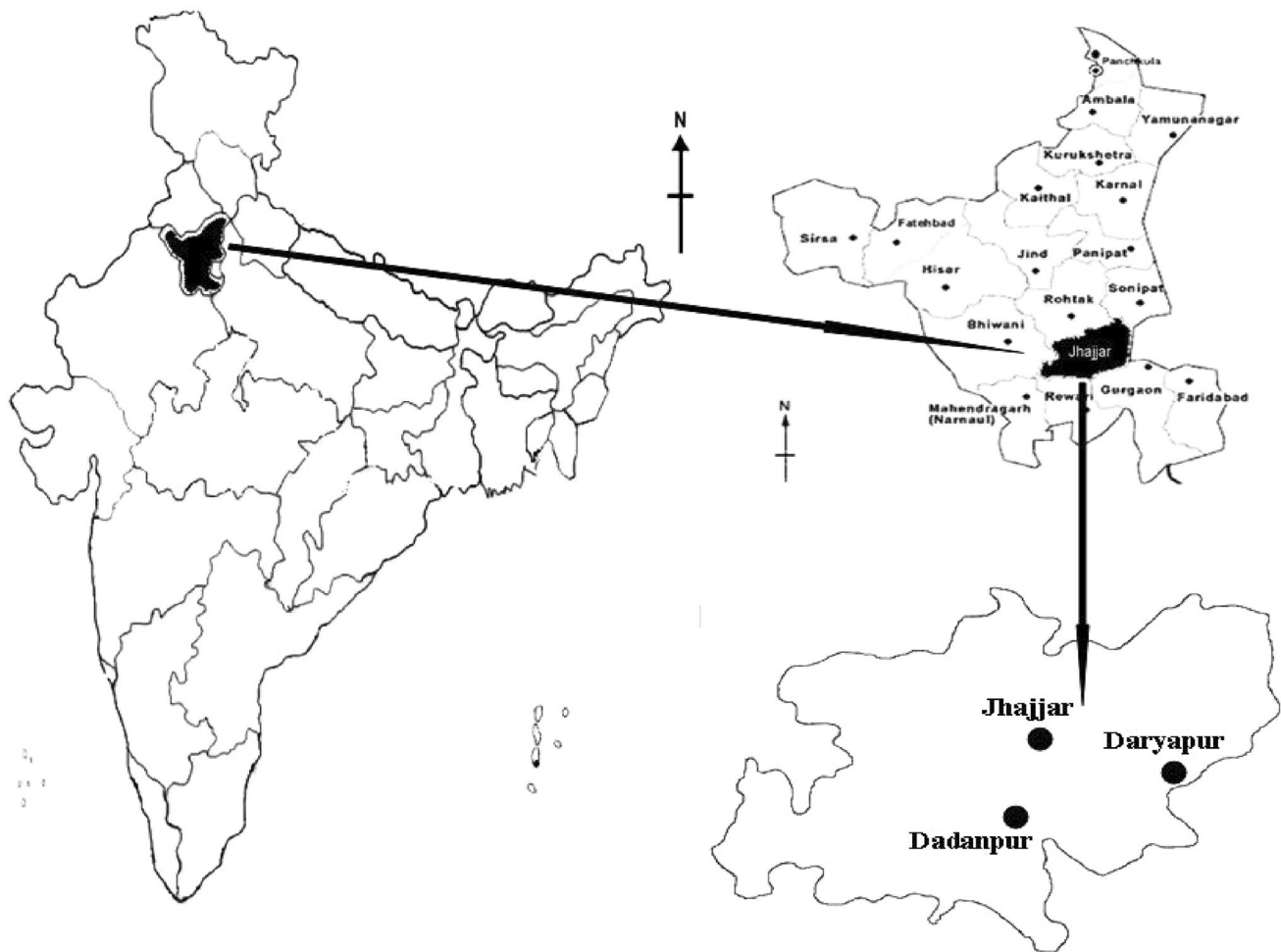
To analyze the level of fluoride in serum, blood samples (5 ml) of 66 children (22 from each location) were drawn intravenously using sterilized needle and syringe and transferred to a dried glass vial at room temperature. After 20 min, the blood was centrifuged at 2000 rpm for 10 min at 4 °C and the supernatant (serum) collected and stored at –20 °C until use. The fluorides in these samples were determined by Orion fluoride ion-specific electrode at IITR, Lucknow.

### Survey on dental fluorosis

The fluorosis data were taken from the school-going children who were residing by birth in the villages to investigate the impact of groundwater fluoride on dental health. A questionnaire, completed with the assistance of the parents, was used to collect information on the personal characteristics (family socioeconomic status and lifestyle), exposure history to fluoride, medical history, etc. pro forma prescribed by Rajiv Gandhi Drinking Water Mission (1993) and earlier described by Dahyia et al. (2000) was used to score the incidence and degree of manifestation of dental fluorosis. The prescribed Tooth Surface Index of Fluorosis (TSIF) was also used, following Jackson et al.'s (1999) method to determine the prevalence of dental fluorosis.

### Statistical analysis

The data were statistically analyzed using the SPSS statistical version 16.0 software. For each set of observations, mean, standard deviation and standard error were calculated.



**Fig. 1** Map showing the location of the study area

## Results

### Fluoride concentration in groundwater, urine and serum

The data of fluoride in drinking groundwater, urine samples and serum samples along with dental fluorosis has been given in Table 1. The mean fluoride level in Jhajar was 2.17 mg/L. The range of fluoride was 1.92–2.60 mg/L. Dadanpur village has the highest mean fluoride value of 2.81 mg/L with a minimum and maximum level of 2.53 and 3.14 mg/L, respectively. The mean fluoride level in Dariyapur village was 2.22 mg/L with a minimum of 1.63 mg/L and maximum level of 3.33 mg/L. It was observed that 100 % samples of drinking groundwater collected from the three locations exceeded the maximum desirable limit of 1 mg/L as laid down by the Bureau of Indian Standard (BIS 1991). The mean fluoride level in the urine samples of individuals of Jhajar, Dadanpur and Dariyapur was 1.51, 1.71 and 1.45 mg/L, respectively.

Similarly, the mean fluoride level in serum samples was 0.15 mg/L in Jhajar, 0.34 mg/L in Dadanpur and 0.17 mg/L in Dariyapur. A significant positive correlation was found between the fluoride content in drinking groundwater and urine ( $r = 0.997$ ), drinking groundwater and serum ( $r = 0.994$ ), and serum and urine (0.949) (Table 2).

### Prevalence of dental fluorosis in young children

To analyze the levels of fluorosis, 842 children were examined. 51.90 % children of Jhajar, 94.63 % of Dadanpur and 36.84 % of Dariyapur village were found to be affected with fluorosis. The stages of dental fluorosis among the individuals were chalky white, yellowish brown and brownish black with horizontal streaks, spots and both spots and streaks. The percentage distribution of dental fluorosis according to TSIF score among affected children is given in Tables 3 and 4. Any examined permanent tooth surface having a TSIF score 0 was defined as not affected with fluorosis. TSIF 1 was defined as fluorosis affected to

**Table 1** Data on fluoride level in water, urine, serum and percentage of fluorosis-affected children

Sites	Fluoride in water (mg/L)	Fluoride in urine samples (mg/L)	Fluoride in serum (mg/L)	%Age of fluorosis-affected children
Jhajjar	2.17 ± 0.09 <sup>a</sup>	1.51 ± 0.13	0.15 ± 0.01	232/447 = 51.90
	0.30 <sup>b</sup>	0.62	0.04	
	1.92–2.60 <sup>c</sup>	0.05–2.64	0.07–0.27	
Dadanpur	2.81 ± 0.08	1.71 ± 0.13	0.34 ± 0.04	194/205 = 94.63
	0.26	0.60	0.21	
	2.53–3.14	0.69–2.80	0.18–1.05	
Dariyapur	2.22 ± 0.20	1.45 ± 0.15	0.17 ± 0.04	70/190 = 36.84
	0.65	0.72	0.18	
	1.63–3.33	0.31–2.50	0.07–0.46	

<sup>a</sup> represents mean ± standard error,

<sup>b</sup> represents standard deviation and

<sup>c</sup> represents minimum and maximum value

**Table 2** The correlation of water, urine, serum fluoride and dental fluorosis

Parameters	<i>r</i> values
Water and urine	0.997*
Water and serum	0.994*
Urine and serum	0.949*
Water and dental fluorosis	0.952*

\* Significant at the 5 % level

some extent. As the TSIF score increased (2–7), the severity of fluorosis among children also increased. TSIF score 4 was most prevalent (Table 4) and was found at all

the places. Children with TSIF score 4 showed staining of tooth enamel that may range from light to very dark brown.

## Discussion

### Occurrence of fluoride in groundwater

Fluoride concentration higher than 1.5 mg/L in drinking water, as prescribed by the WHO, results in dental and skeletal fluorosis, and renal and neuronal disorders along with myopathy (Ayoob and Gupta 2006; Das and Nag 2016; Narsimha and Sudarshan 2016). In the present study,

**Table 3** Data on the incidence and severity of dental fluorosis in school children

Sites	No. of individuals examined	No. of affected individuals	Stages of dental fluorosis			Types of dental fluorosis		
			Chalky white	Yellowish brown	Brownish black	Horizontal streaks	Spots	Both
Jhajjar	447	232 (51.90)	110 (24.61)	98 (21.93)	24 (5.37)	90 (20.13)	121 (27.07)	21 (4.70)
Dadanpur	205	194 (94.63)	73 (35.61)	101 (49.27)	20 (9.75)	75 (36.58)	48 (23.41)	71 (34.63)
Dariyapur	190	70 (36.84)	12 (6.31)	45 (23.68)	13 (6.84)	21 (11.05)	40 (21.05)	9 (4.74)

Figures in parenthesis represent the percentage

**Table 4** Percent distribution of fluorosis in children by TSIF (number of individuals in parenthesis)

Sites	TSIF score						
	N	0	1	2	3	4	5–7
Jhajjar	447	48.10 (215)	4.47 (20)	8.95 (40)	11.18 (50)	21.93 (98)	5.37 (24)
Dadanpur	205	5.36 (11)	9.76 (20)	12.19 (25)	13.11 (27)	49.27 (101)	10.31 (21)
Dariyapur	190	63.16 (120)	0 (0)	0 (0)	6.31 (12)	23.68 (45)	6.84 (13)

Number given in parenthesis represents the number of individuals

geological sources and fertilizer used in agriculture are the possible contributors of fluoride in groundwater, and industrial input is absent due to the rural setting. The study area consists of alluvial plains with sand, silt and clay and kankar mixed in different proportions. Jhajjar District is a part of the Dugan ethnic plain ranging from Pleistocene to recent in age, and Aeolian deposits of sub-recent age cap the plains. The soil texture comprises loam, yellowish and brown in color. The geological formation of Gurgaon District, which is the adjacent district to the study area, has been described by Singh (1996). He demonstrated that the Aravalli system is the oldest formation available in Gurgaon District and composed of quartzite sandstone, mica schists, phyllites, silica sand, china clay, ordinary sand, crystalline limestone, etc. The population of the study area depends on the groundwater for drinking purpose. The mean values of fluoride levels at all the three locations were found to be more than the prescribed value of 1 ppm (WHO 1996).

Drinking water consumption by the human body totally depends on the atmospheric temperature. Extremely hot conditions were present in the study area during the summer season and the temperature ranged from 25 to 48 °C. The optimal concentration of fluoride in drinking water for regions with extreme climates should be 0.5–0.7 mg/L, as prescribed by World Health Organization (Murray 1986). A study conducted by Singh et al. (2007) in Gurgaon District, adjacent to the study area, revealed that the water consumption was approximately 2.5 L per child per day during the months of April to August. They observed that the fluoride intake by children from ingested water was 4.20, 4.45 and 8.05 mg fluoride/person/day at Pataudi, Harsaru and Haily Mandi villages, respectively, whereas in the present study fluoride intake by children from ingested water was 5.44, 7.05 and 5.57 mg fluoride/person/day at Jhajjar, Dadanpur and Dariyapur villages, respectively with 2.5 L drinking water consumption per child per day. The results of fluoride ingestion are closely related in both the studies. Yadav et al. (2007) suggested that absorption of fluoride from water ranges from 86 to 97 %. The Central Ground Water Board (2007) also reported a higher groundwater fluoride level in Jhajjar District ranging from 1.73 to 5.94 mg/L and recommended that groundwater consumption was harmful for humans. The highest groundwater fluoride concentration from Haryana State was 48 mg/L in Rewari District (UNICEF 1999), adjacent district to the study area. The dietary fluoride intake recommended by the National Research Council (1989) is in the range of 0.1–1.0 mg/person/day for children under the age of 1 year; 0.5 to 1.5 mg/person/day for children between 1 and 3 years; up to 2.5 mg/person/day for children under 12 years and 1.5–4.0 mg/person/day for adults. During the

study, only drinking water-ingested fluoride was calculated, which was higher than the recommended dietary fluoride limit for adults. It is evident from the results that the population in the study area is chronically exposed to higher levels of fluoride from drinking water. Various studies from different parts of the world showed the excess of fluoride in groundwater: Sri Lanka (Disanayake 1991), Serbia (Mandinic et al. 2010), China (Chen et al. 2012), Ethiopia and Malawi (Msonda et al. 2007). Excessive groundwater fluoride level was also reported in several states of India: Rajasthan (Choubisa 1997), Tamil Nadu (Gopalakrishnan et al. 1991), Uttar Pradesh (Jha et al. 2010), Bihar (Verma et al. 2016), Chhattisgarh (Patel et al. 2015) and Haryana (Singh et al. 2007; Meenakshi et al. 2004; Yadav and Lata 2003).

### Relationship between dental fluorosis, urine and serum fluoride

Three sites were screened to conduct the survey on the prevalence of dental fluorosis based on the higher fluoride concentration in groundwater. Nearly 50 % of the absorbed fluoride in each day becomes associated with calcified tissues within 24 h and the remainder is excreted in the urine (Neuman and Neuman 1958). In plasma, fluoride is transported as ionic fluoride and non-ionic fluoride. Ionic fluoride does not bind to plasma proteins and is easily excreted with the urine (Singh et al. 2007). Urinary fluoride clearance increases with urine pH due to a decrease in the concentration of HF (IPCS 2002). Other studies have also shown that the uptake of fluoride by the skeleton is most efficient in children and decreases with age (Whitford 1999; Ozsvath 2009; Viswanathan et al. 2009). The present study revealed that the prevalence of dental fluorosis increases with increase of groundwater fluoride concentration. In Dadanpur village 94.63 % surveyed children were affected by dental fluorosis, out of which 49.27 % were suffering from yellowish brown stage of dental fluorosis and 36.58 % from horizontal streak type of dental fluorosis. Statically positive significant correlation ( $r = 0.952$ ) occurs between groundwater fluoride and dental fluorosis. Other studies also showed that dental fluorosis increases with fluoride concentration in drinking water (Viswanathan et al. 2009; Mandinic et al. 2010; Rango et al. 2012). The fluoride level in biomaterials such as urine and blood has been proposed as the most reliable indicator of exposure to fluoride (Kokot and Drzewiecki 2000). The generally accepted fluoride concentration in serum is 0.15 ppm (Singer and Armstrong, 1965). The acceptable concentration of urine fluoride is 1 mg/L (Jaganmohan et al. 2010). The mean serum fluoride levels were higher than the accepted values at two locations (Dadanpur and Dariyapur). However, the mean serum



**Table 5** Literature data on serum, urine and water fluoride concentration

	Serum F $\mu\text{g/L}$	Urinary F $\mu\text{g/L}$	Water F $\mu\text{g/L}$	Locations
Husdan et al. ( $n = 136$ )	9.5–43.7	No results	No results	Toronto
Parkins et al. ( $n = 41$ ) (optimal fluoridated water)	19–112	No results	No results	Iowa City
Shimonovitz et al. ( $n = 20$ )	$30.3 \pm 15$	No results	0.22–0.49	Jerusalem
Abdennebi et al.	No results	170–47,500	1003	Youssoufia city
Torra et al. ( $n = 250$ )	1–45	159–1990	200	Barcelona
Singh et al. ( $n = 400$ )	No results	900–3250	950–5200	Gurgaon
Jaganmohan et al. ( $n = 90$ )	1470–2270	1070–4000	2370–6740	Nellore
Present study	70–1050 ( $n = 66$ )	50–2800 ( $n = 100$ )	2400 ( $n = 30$ )	Jhajjar

fluoride value at Jhajjar ranged from 0.07 to 0.27 mg/L with a mean value of 0.15 mg/L. Fluoride in urine samples from the study area sites was higher than the prescribed accepted level of 1 mg/L. Elevated concentration in urine supported a clinical diagnosis of fluorosis (Nayak et al. 2009).

The significant positive correlation between the fluoride contents of drinking groundwater, urine, serum and prevalence of dental fluorosis among the sampled children is consistent with the findings of Xiang et al. (2004) and suggest that fluorosis among the investigated village children population is mainly caused by fluoride in drinking groundwater. As these children were residing at these places since birth, they were exposed to fluoride in drinking groundwater from the time of permanent teeth formation and enamel maturation, reflecting dental fluorosis.

The various studies on fluoride level (Parkins et al. 1974; Husdan et al. 1976; Shimonovitz et al. 1995; Abdennebi et al. 1995; Torra et al. 1998; Singh et al. 2007; Jaganmohan et al. 2010) in groundwater, serum and urine have been shown in Table 5. The present investigation indicates that fluoride concentrations in serum were higher than those in earlier studies, except fluoride reported by Jaganmohan et al. (2010). Similarly, urinary fluoride concentration was also higher, except fluoride reported by Abdennebi et al. (1995); Singh et al. (2007) and Jaganmohan et al. (2010).

## Conclusion

Fluoride when taken in controlled quantities reduces tooth decay. But excess fluoride ingested through all sources adversely affects the appearance of children's teeth during development and causes dental fluorosis. The ingested fluoride level in children in the study area was higher than the recommended dietary fluoride limit for children given by the National Research Council. The present study revealed that the underground drinking water of the investigated region was contaminated with fluoride, and the population of the

study area was chronically exposed to higher levels of fluoride from drinking water. There was a significantly positive correlation between the fluoride content of groundwater, urine and serum of fluorosis-affected children. This suggests that a high level of fluorosis in the study area was due to the consequence of a higher concentration of fluoride in underground aquifers. A maximum number of fluorosis-affected children (94.63 %) were recorded from Dadanpur village. The study also revealed that the fluoride level in urine and serum was also higher than the accepted level. Thus, there is a need to be aware of the removal of excess fluoride from drinking water.

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