Dental fluorosis, nutritional status, kidney damage, and thyroid function along with bone metabolic indicators in school-going children living in fluoride-affected hilly areas of Doda district, Jammu and Kashmir, India

Arjun L. Khandare · Shankar Rao Gourineni · Vakdevi Vālendandi

Received: 16 June 2017 / Accepted: 5 October 2017 © Springer International Publishing AG 2017

Abstract A case-control study was undertaken among the school children aged 8–15 years to know the presence and severity of dental fluorosis, nutrition and kidney status, and thyroid function along with bone metabolic indicators in Doda district situated at high altitude where drinking water was contaminated and heat stress. This study included 824 participants with an age of 8–15 years. The results of the study revealed that dental fluorosis was significantly higher in affected than control area children. Urinary fluoride was significantly higher ($p < 0.05$) in affected children as compared to the control area school children. Nutritional status of affected children was lower than control area children. The chronic kidney damage (CKD) was higher in affected than control school children. Thyroid function was affected more in affected than control area schools. Serum creatinine, total alkaline phosphatase, parathyroid hormone, 1, 25(OH)$_2$ vitamin D, and osteocalcin were significantly higher ($p < 0.05$) as compared to control school children, whereas there was no significant difference in triiodothyronine (T3), thyroxine (T4), and 25-OH vitamin D among the two groups. There was a significant decrease in thyroid-stimulating hormone (TSH) in the affected area school children compared to control. In conclusion, fluorotic area school children were more affected with dental fluorosis, kidney damage, and some bone indicators as compared to control school children.

Keywords Fluoride · Urinary fluoride · Dental fluorosis · Glomerular filtration rate · 1,25(OH)$_2$ vitamin D

Introduction Fluoride (F) intake in permissible limit (< 1 ppm) through water prevents the formation of dental caries (Petersen and Lennon 2004). However, exposure to high F (>1 ppm) can generate several alterations. Chronic fluoride poisoning is a worldwide health problem that occurs in endemic areas where the fluoride content in drinking water is above the optimal level (> 1 ppm) (Xiang et al. 2010). The World Health Organization (WHO) referred to fluoride above 1.0–1.5 mg/L in drinking water, especially drawn from the groundwater of areas of granite rock, result in pathological changes in teeth causing dental fluorosis (DF), which is categorized by light yellow to brown-black horizontal lines on the teeth surface and chipped off edges (Pérez-Pérez et al. 2017). It is known that high intake of F for long time produces bone damage and kidney damage and lowers the intelligent quotient (IQ) in children (Das and Mondal 2016; Ranjith 2015; Qin et al. 2009).

The high-risk areas of F concentrations are mostly located in arid and semi-arid regions that are characterized by a rapid rate of chemical weathering of
geological resources (Rango et al. 2012). In the human body, the total fluoride ingested remains for a long time; however, approximately 80% of fluoride is excreted mainly through urine; the rest of it is absorbed into body tissues from where it is released very slowly (WHO 1996). The excreted fluoride through urine can be considered as biomarkers of fluoride and it serve to identify deficient or excessive consumption and bioavailability of fluoride in the body (Watanable et al. 1994). Among the various biomarkers of fluoride exposure, the urinary fluoride (UF) was considered to be the best indicator of fluoride exposure because it can be collected noninvasively and systematically reflects the burden of fluoride exposure through all the sources (diet and water). Hence, special attention has been given to it as a biomarker, and is used as an indirect indicator of fluoride exposure.

There is a limited data on the prevalence and severity of fluorosis in the hilly and disturbed area of Doda district, Jammu and Kashmir, India. There is no systemic study on nutritional status, dental fluorosis, kidney, and thyroid and bone metabolic indicators in the area of Doda district, Jammu and Kashmir, affected with fluorosis. Hence, the present descriptive study has been undertaken to plan appropriate intervention strategies in the future.

Materials and methods

Study design

The detailed case-control study was undertaken to cover 10 schools in Doda district of Jammu and Kashmir out of which 8 rural (affected; $F = 1.43$ to 3.84 mg/L) and 2 urban (control; $F = <1$ mg/L). The school-going children were divided into 2 categories: control and affected based on their drinking water fluoride levels.

The Doda district lies within latitude $32°25′00″$ and $34°14′00″$ N and $75°00′$ and $76°45′30″$ E and has an average elevation of 1107 m (3631 ft). This case-control study has been conducted in an endemic fluorosis area of Jammu and Kashmir State where geogenic concentration of F in groundwater was high (1.153 to 27.216 mg/L) (Inder et al. 2000) and known to be endemic for dental fluorosis (Thakuria 2007).

All the participants were born and had resided in the area since their birth. An appropriate questionnaire was administered to all the participants at the time of admission to collect demographic data. Diagnosis of dental fluorosis was performed by clinical examination through oral examination of each student, by a dental specialist (from local hospital) in the common room of the school, with the subject seated in a chair in bright daylight. The dental specialist used a mirror and a sterile dental probe for oral examination. The presence and severity of dental fluorosis were recorded. The Dean index was used to determine the grade of dental fluorosis (Dean 1942) which was selected because of its accuracy to identify DF severity. Anthropometric measures such as weight and height were recorded, and body mass index (BMI) was calculated. Water samples from all drinking water sources were collected from the selected control and affected villages in clean, high-density polyethylene bottles. First morning spot urine samples were collected in polyethylene containers and stored, adding 2 to 4 drops of toluene as a preservative and transported to laboratory, NIN, Hyderabad and stored at 4 °C till analysis.

Twelve-hour fasting blood samples were collected in a serum vacutainer (Vacuette, Greiner Bio-one, Austria). Serum was separated by centrifugation at 3000 rpm for 20 min and stored in an ice-lined refrigerator (ILR) at 4 °C in the district hospital. The samples were transferred to the laboratory at NIN, Hyderabad in cold condition using vaccine carrier and kept at $-80$ °C until further analysis.

Biochemical parameters in serum

Serum samples were analyzed for biochemical parameters such as creatinine and alkaline phosphatase (ALP) using ACE Alera Auto analyzer, Alfà Wassermann, Inc., USA. Serum Osteocalcin was analyzed using “Micro Vue” Osteocalcin Immune Assay kit supplied by Quidel Corporation, USA. Parathyroid hormone (PTH), 25-OH and 1, 25 (OH)$_2$ vitamin D, and T3 and T4 levels in serum were measured by using DiaSorin kits supplied by DiaSorin Minnesota, USA. Thyroid-stimulating hormone (TSH) was measured by Immunoradiometric assay (IRMA) kit, DiaSorin, Saluggia (VC) Italy.

Drinking water and UF concentrations were analyzed using fluoride-specific ion electrode, (Orion 9609) (Tusl 1970) which was calibrated with fresh, serially diluted standard solutions. During the measurement, ionic strength buffer solution was added to each sample for analysis.
The BMI was calculated by the formula,

$$\text{BMI} = \frac{\text{weight in kg}}{\text{height in meter}^2}$$

The status of kidney damage was assessed by glomerular filtration rate (GFR) and it was calculated by the Bedside Schwartz Formula (Schwartz et al. 1976),

$$\text{GFR (mL/min/1.73 m}^2) = \frac{(0.41 \times \text{height in cm})/\text{creatinine in mg/dL}}{\text{}}.$$ 

Written informed consent was obtained from all the participants or by their school Head Master in case they were minors. All data were managed to ensure the protection of individual rights and maintain confidentiality. This study was approved by the Institutional Human Ethics Committee, National Institute of Nutrition, Hyderabad, India (Protocol No. 18/2013/1).

Statistical analysis

Results are presented as means and standard deviations between the observed values. Relationships between water fluoride level with prevalence and severity of fluorosis, fluoride exposure dose, were assessed by linear regression analysis. The statistical differences between the bone metabolic indicators of control and affected group children were quantitatively assessed by the statistical parameters such as correlation coefficient ($r$), Fisher-ratio (F-ratio), and one-way ANOVA. All statistical analyses were performed by Microcal-Origin version 6. $p < 0.05$ was considered as statistically significant.

Results

Prevalence of dental fluorosis

The study population consisted of 824 participants with 379 from affected area [boys 193 (50.9%); girls 186 (49.1%)] and 445 from control area [boys 301 (67.6%); girls 144 (32.3%)]. Out of 379 from affected area, 48% were affected by different grades of dental fluorosis; boys and girls were equally affected. Out of 8 affected schools, 2 schools belong to Malwas and Golibagh having high drinking water fluoride (> 3 ppm). In these village schools, more than 95% students were affected with different grades of dental fluorosis (out of 33 students 32 were affected, 12 in grade I, 4 in grade II, 8 in grade III, and 8 were in grade IV) (Fig. 1). Two schools named Green Model School and Govt. Higher Secondary School having average drinking water fluoride 1.13 ppm (0.32–1.18 ppm) were considered as control schools. Out of 445 students examined for clinical signs and symptoms of dental fluorosis, only 56 were affected (12.5%); most of them were in grade I and 6 students were in grade II. The community fluorosis index (CFI) for affected and control area was calculated and given in Table 1. There were no significant differences observed in the extent of prevalence and severity of dental fluorosis among the boys and girls ($p < 0.680$) in affected areas (Table 1). Figure 2a–d illustrates that the increase in water fluoride levels in study areas quantitatively enhances the fluoride exposure dose, prevalence of fluorosis, and CFI values, respectively.

Nutritional status

To know the nutritional status, the BMI was calculated and it was ranged from 14.59 to 18.70 for the age groups of 6 to 15 years in boys of affected area, whereas 15.87 to 18.71 for same age groups of control area. However, affected area boys aged 11 and 12 years BMI was 16.15 and 16.28, which was low as compared to control area boys (BMI = 17.03 and 17.49). BMI for girls from affected and control schools ranged from 15.40 to 20.47 for the age groups 6 to 15 years, whereas 15.37 to 19.74 for same age groups from control area.
Table 1  Water fluoride levels, prevalence of fluorosis, and community fluorosis index (CFI) in normal and high fluoride areas

<table>
<thead>
<tr>
<th>Areas</th>
<th>Water fluoride level (mg/L)</th>
<th>Gender</th>
<th>No. of children classified according to Dean’s classification</th>
<th>Prevalence of fluorosis (%)</th>
<th>CFI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control area</td>
<td>1.13 ± 0.42</td>
<td>Boys</td>
<td>220 45 20 11 5 0 0</td>
<td>27 0.23</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Girls</td>
<td>99 24 16 4 1 0 0</td>
<td>31 0.25</td>
<td></td>
</tr>
<tr>
<td>High fluoride areas</td>
<td>1.85 ± 0.25</td>
<td>Boys</td>
<td>26 0 3 2 0 0 1</td>
<td>19 0.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Girls</td>
<td>21 0 2 0 1 0 1</td>
<td>16 0.32</td>
<td></td>
</tr>
<tr>
<td>Amora</td>
<td>2.04 ± 0.49</td>
<td>Boys</td>
<td>4 2 6 3 1 0 0</td>
<td>75 0.84*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Girls</td>
<td>6 0 4 2 2 0 0</td>
<td>57 0.79*</td>
<td></td>
</tr>
<tr>
<td>Ghat</td>
<td>2.05 ± 0.51</td>
<td>Boys</td>
<td>71 7 21 12 9 5 1</td>
<td>44 0.63*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Girls</td>
<td>64 15 17 17 13 6 1</td>
<td>52 0.74*</td>
<td></td>
</tr>
<tr>
<td>Malvas</td>
<td>3.12 ± 0.10</td>
<td>Boys</td>
<td>0 0 1 1 0 3 1</td>
<td>100 2.58*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Girls</td>
<td>1 0 2 0 2 0 6</td>
<td>91 2.73*</td>
<td></td>
</tr>
<tr>
<td>Golibagh</td>
<td>3.84 ± 0.01</td>
<td>Boys</td>
<td>0 1 1 2 2 2 4</td>
<td>100 2.54*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Girls</td>
<td>0 0 0 1 0 1 2</td>
<td>100 3.13*</td>
<td></td>
</tr>
</tbody>
</table>

*Statistically significant ($p < 0.05$)

Fig. 2  a–d Linear correlations between water fluoride levels with fluoride exposure dose (a), prevalence of fluorosis (%) (b) and CFI (c), and relationship between CFI and fluoride exposure dose (d)
Blood and urinary parameters

Fluoride exposure was significantly higher ($p < 0.05$) in the affected area school children than the control area (Table 2). The UF was significantly higher in the affected area school children than the control. Creatinine, alkaline phosphatase, osteocalcin, parathyroid hormone, and 1, 25-(OH)$_2$ vitamin D in the affected area school children were significantly higher than the controls (Tables 2 and 3). There was a significant reduction ($p < 0.001$) in GFR in the affected area school children (30 units less) than the control (Table 2). In both the areas, serum 25-OH vitamin D was lower than the normal range. Serum TSH levels in the affected area school children were significantly lower than the control area children ($p < 0.02$). There was no significant difference in serum T3 ($p = 0.9731$) and thyroxine (T4) level ($p = 0.103$) in control and affected school children (Table 4).

Discussion

According to the prevalence and severity of fluorosis based on the CFI levels (> 0.6), fluorosis is considered to be a public health problem in affected villages (Arnora, Ghat, Malvas, and Golibagh) of Doda district (Table 1). An average of 77% prevalence of dental fluorosis was observed among the children where the fluoride level was 2 to 4 ppm in affected villages. As per the regression analysis, an increase of 1 mg/L fluoride level in drinking water consequently increases the fluoride exposure dose at about 0.1 mg/kg/d which elevates the extent of prevalence of fluorosis about 31% among the children in study areas of Doda district (Fig. 2a–d). However, the fluoride level in drinking water of Doda town was less than the permissible limit (< 1.5 mg/L); however, there was a considerable prevalence of fluorosis (29%) and this may be due to black tea consumption, known to contribute fluoride (Waugh et al. 2016). The extent of prevalence of fluorosis in Doda district (high altitude) was considerably higher when it was compared with the plain areas with similar drinking water fluoride levels which was also reported elsewhere (Hou et al. 2014; Kececi et al. 2014). This may be due to the alteration in acid-base balance caused by hypobaric hypoxia among the residents in high-altitude areas, which increase the retention of fluoride in body due to lesser excretion through urine which enhances the risk of fluorosis which was also reported by Whitford (1999).

In the present study, we found there was no much difference in BMI of the school children from control and affected areas. However, the reports available in the literature contradict with each other. A positive correlation was observed between BMI and drinking water fluoride levels (Das and Mondal 2016). In another study conducted in Delhi, India, found there was no significant association of disease with gender, source of drinking water, and with BMI (Tiwari et al. 2010).

In the present study, UF levels of affected children was 3.28 ± 1.71 mg/L, whereas water fluoride level ranged from 1.5 to 4.0 mg/L was comparable with the previous studies conducted by Ding et al. (2011). Kidney is a site of active excretion and excretes 50–80% of fluoride from all sources of exposure (Spencer et al. 1969). High UF in the affected group of children reflects high exposure of fluoride (drinking water and tea). In other Indian studies, of aged 6 to 18 years, the highest UF concentration reported was 17 mg/L when fluoride water concentration was of 2.11 mg/L (Das and Mondal 2016) whereas in other study, individuals aged 11–16 years, an average UF was 2.35 mg/L (Singh et al. 2007). These variations might be due to different use and consumption practices of water and other sources of fluoride like tea and black salt among populations.

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Fluoride exposure dose (mg/kg/d)</th>
<th>Urinary fluoride level (mg/L)</th>
<th>Serum creatinine (mg/dL)</th>
<th>GFR (mL/min/1.73m$^2$)</th>
<th>CKD* stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.05 ± 0.01</td>
<td>1.91 ± 0.64</td>
<td>0.45 ± 0.16</td>
<td>120.9 ± 27.4</td>
<td>Normal</td>
</tr>
<tr>
<td>Affected</td>
<td>0.09 ± 0.02†</td>
<td>3.28 ± 1.71†</td>
<td>0.85 ± 0.35†</td>
<td>84.1 ± 33.1†</td>
<td>Stage 2</td>
</tr>
<tr>
<td>Normal range</td>
<td>0.05–0.07</td>
<td>≤ 1.5</td>
<td>0.5–1.2</td>
<td>≥ 90</td>
<td>Normal</td>
</tr>
</tbody>
</table>

*Chronic kidney disease
† Statistically significant ($p < 0.05$)
Previous studies demonstrated that the intake of excess fluoride can enhance the occurrence of renal failure by damaging tubular epithelial cells in the kidney leading to chronic kidney diseases (Juncos and Donadio 1972; Ludlow et al. 2007; Xiong et al. 2007). In the present study, a significant increase in serum creatinine due to a considerable reduction in GFR in affected children indicates damage of the kidney. According to the GFR level (< 90 mL/min/1.73m²) estimated among the affected children indicated that they are in the second-stage chronic kidney diseases. This may be due to higher altitude, heat stress, and contaminated water in the area which has been reported in earlier studies (Lunyera et al. 2016). The impaired kidney function further enhances the retention of fluoride in the body and increases the risk of fluorosis which correlates with earlier study (Xiong et al. 2007).

It is known that the excess fluoride in drinking water elevates serum alkaline phosphatase and osteocalcin, in turn, increases osteoblastic activity and bone formation in children in high-fluoride endemic areas (Johnson et al. 1979; Khandare et al. 2005; Ross and Knowlton 1998). The same was observed in the present study in the affected area children indicating the enhanced osteoblastic activity which may increase fracture risk.

Earlier study has demonstrated elevated levels of PTH (Khandare et al. 2005) in the fluoride endemic areas, the same holds good in the present study also. The increase in PTH may be due to maintain the calcium (Ca) homeostasis in blood (Gupta et al. 2001). In India, Ca deficiency is rampant irrespective of fluorotic or non-fluorotic areas. In fluorotic area, there is an additional demand of Ca because of false bone formation which also shows increase in alkaline phosphatase and osteocalcin in the present study. To increase the absorption of Ca at the intestine, the conversion of 25-hydroxy vitamin D to 1,25-dihydroxy vitamin D was increased (Shankar et al. 2013). In addition, lesser exposure to sunlight at high-altitude areas may create the deficiency of vitamin D level (25-hydroxy vitamin D) (Dusso et al. 2011); however, the same was not seen in the present study. The increased 1,25-dihydroxy vitamin D in the present study might be to maintain the Ca homeostasis.

Excess F exposure in the high-altitude area children (commonly deficient of iodine) elevates the risk of hypofunction of thyroid glands (Mordes et al. 1983; Rastogi et al. 1977). The significantly decreased levels of TSH in the affected children may be due to the excess F intake. The excess F intake which competitively affects the iodine absorption might have inhibited its activities in thyroid glands to release TSH. Additionally, the excess F affects the catabolism (deiodination process) of conversion from T4 to T3, leads to a deficiency in T3 (the active form of thyroid hormone) (Singh et al. 2014). In the present study, the deiodination of T4 to T3 might have been inhibited by F which is indicated by the low levels

---

**Table 3** Levels of alkaline phosphatase, osteocalcin, PTH, 25-(OH)-vitamin D, and 1, 25 (OH)₂ vitamin D in serum of control and affected group of children (values represent mean±standard deviation)

<table>
<thead>
<tr>
<th>Group</th>
<th>Osteocalcin (ng/mL)</th>
<th>Parathyroid hormone (PTH) (pg/mL)</th>
<th>Alkaline phosphatase (IU/L)</th>
<th>25-(OH) vitamin D (ng/mL)</th>
<th>1, 25-(OH)₂ vitamin D (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.7 ± 3.3</td>
<td>27 ± 5</td>
<td>266 ± 70</td>
<td>13.0 ± 7.6</td>
<td>98.8 ± 38.1</td>
</tr>
<tr>
<td>Affected</td>
<td>31.3 ± 13.2*</td>
<td>49 ± 12*</td>
<td>401 ± 125*</td>
<td>9.8 ± 5.9</td>
<td>146.2 ± 38.8*</td>
</tr>
<tr>
<td>Normal range</td>
<td>9.0–42.0</td>
<td>13–54</td>
<td>98–279</td>
<td>30–74</td>
<td>24–86</td>
</tr>
</tbody>
</table>

*Statistically significant (p < 0.05)

**Table 4** Levels of thyroxine (T₄) and triiodothyronine (T₃), thyroid-stimulating hormone (TSH) in serum of control and affected group of children (values represent mean±standard deviation)

<table>
<thead>
<tr>
<th>Group</th>
<th>Thyroxine (T₄) (μg/dL)</th>
<th>Triiodothyronine (T₃) (ng/mL)</th>
<th>Thyroid-stimulating hormone (TSH) (mU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.9 ± 1.6</td>
<td>0.68 ± 0.35</td>
<td>3.4 ± 0.5</td>
</tr>
<tr>
<td>Affected</td>
<td>16.1 ± 2.9</td>
<td>0.63 ± 0.24</td>
<td>2.9 ± 0.6*</td>
</tr>
<tr>
<td>Normal range</td>
<td>6.1–11.8</td>
<td>0.8–2.0</td>
<td>0.3–4.0</td>
</tr>
</tbody>
</table>

*Statistically significant (p < 0.05)
of T3 in affected area children compared to control. These thyroid hormone disturbances among the fluorosis-affected children show the vulnerability to thyroid hormone deficiency created health risks such as low IQ, deaf-mutism, and cretinism in children have been reported earlier (Saxena et al. 2012; Susheela et al. 2005).

Conclusion

Increase in water fluoride levels in study areas enhances the fluoride exposure dose, prevalence of fluorosis, CFI, urinary F, alkaline phosphatase, osteocalcin, PTH, and TSH. There was no significant difference in nutrition status from control and affected children; however, significant increase in serum creatinine was observed and may be due to a considerable reduction in GFR in affected children indicating partial damage of the kidney. The increase in PTH and 1,25-(OH)2 vitamin D shows Ca deficiency in affected area than the controls. The fluoride altered thyroid hormone levels in the affected area children compared to controls. To protect the health of the young children in affected hilly areas of Doda district, the immediate intervention with safe drinking water supply is advised.

Acknowledgements The authors thank the “Indian Council of Medical Research” for funding the study. The authors also acknowledge the encouragement and guidance of the director-in-charge, National Institute of Nutrition.

References


Rastogi, G. K., Malhotra, M. S., Srivastava, M. C., et al. (1977). Study of the pituitary-thyroid functions at high altitude in


