

Association of dental and skeletal fluorosis with calcium intake and serum vitamin D concentration in adolescents from a region endemic for fluorosis

Prerna P. Patel, Pinal A. Patel, M. Mughal Zulf¹, Bhrrugu Yagnik², Neha Kajale³, Rubina Mandlik³, Vaman Khadilkar³, Shashi A. Chiplonkar³, Supriya Phanse³, Vivek Patwardhan³, Priscilla Joshi⁴, Ashish Patel, Anuradha V. Khadilkar³

Department of Biotechnology, Hemchandracharya North Gujarat University, Patan, ²Department of Biochemistry, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, ³Department of Growth and Pediatric Endocrine Unit, Hirabai Cowasji Jehangir Medical Research Institute, Jehangir Hospital, ⁴Department of Radiology, Bharati Vidyapeeth Medical College, Pune, Maharashtra, India, ¹Department of Paediatric Endocrinology, Royal Manchester Children's Hospital, Manchester, United Kingdom

ABSTRACT

Context: Fluorosis is controlled by the duration of fluoride exposure and calcium and Vitamin D nutrition status. **Aim:** To examine (a) prevalence of dental and skeletal fluorosis in adolescents from upper, middle, and lower socioeconomic strata (SES) and (b) association of fluorosis with calcium intake and Vitamin D status. **Settings and Design:** A cross-sectional study conducted in 10–13.9 years apparently healthy adolescents ($n = 90$), from different SES of Patan (Gujarat, India). **Materials and Methods:** Dental fluorosis was graded as mild, moderate, and severe. Radiographs of the right hand and wrist were examined and graded. Serum 25 hydroxyvitamin D₃ (25OHD) and parathyroid hormone concentrations were measured. Diet was recorded (24 h recall) and calcium intake was computed (C-diet V-2.1, 2013, Xenios Technologies Pvt. Ltd). **Statistical Analysis:** Generalized linear model was used to analyze relationships between fluorosis, SES, serum 25OHD concentration, and calcium intake. **Results:** Fluorosis was predominant in lower SES (17% had both dental and radiological features whereas 73% had dental fluorosis); no skeletal deformities were observed. Mean 25OHD concentrations and dietary calcium were 26.3 ± 4.9 , 23.4 ± 4.7 , and 18.6 ± 4 ng/ml and 441.2 ± 227.6 , 484.3 ± 160.9 , and 749.2 ± 245.4 mg/day, respectively, for lower, middle, and upper SES ($P < 0.05$). Fluorosis and SES showed a significant association (exponential $\beta = 2.5$, $P = 0.01$) as compared to upper SES, middle SES adolescents were at 1.3 times while lower SES adolescents were at 2.5 times higher risk. Serum 25OHD concentrations ($P = 0.937$) and dietary calcium intake ($P = 0.825$) did not show a significant association with fluorosis. **Conclusion:** Fluorosis was more common in lower SES adolescents, probably due to the lack of access to bottled water. Relatively adequate calcium intake and serum 25OHD concentrations may have increased the efficiency of dietary calcium absorption, thus preventing severe fluorosis.

Key words: 25 hydroxyvitamin D₃, adolescents, calcium, dental fluorosis, skeletal fluorosis

INTRODUCTION

High fluoride concentrations in ground water (above

1.5 mg/L) may pose health problems; in India, high fluoride levels are found in certain geographical regions. Patan (Northern Gujarat, western India) is one of the affected districts where fluoride concentrations in water are beyond the permissible limit (>1.5 mg/L), and hence the area is endemic for fluorosis.^[1] Fluoride has been detected

Corresponding Author: Dr. Anuradha V. Khadilkar, Hirabai Cowasji Jehangir Medical Research Institute, Block 5, Lower Ground Floor, Jehangir Hospital, 32, Sassoon Road, Pune - 411 001, Maharashtra, India. E-mail: anuradhavkhadilkar@gmail.com

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as well as quantified in Patan revealing high fluoride concentration in ground and surface water (>1.5 ppm).^[1,2] Fluoride concentration in ground water in Patan varies from 1.96 to 10.85 ppm.^[3] Although fluoride consumption within recommended safe limits (0.05–0.07 mg F/Kg/day) prevents dental caries, increase in its intake can lead to the development of fluorosis.^[4,5]

Fluoride has a strong affinity for hard tissues and easily gets deposited in teeth and bones in the body. Thus, chronic exposure to elevated levels of fluoride leads to dental and skeletal fluorosis.^[5,6] Dental fluorosis occurs due to exposure to fluoride during mineralization; fluoride replaces the hydroxyapatite crystals in teeth and gets deposited as fluorapatite crystals, which becomes clinically visible as dental mottling. Mottling of the teeth is usually seen in younger children who are developing their front teeth.^[4,6] Excessive fluoride also replaces the hydroxyl groups present in the hydroxyapatite crystal of the bone, forming fluorapatite. This has large crystal size due to which the bone becomes brittle and susceptible to fracture risk.^[7,8] Moreover, young bones retain more fluoride than older bones.^[5] Clinical manifestations of fluorosis may be aggravated by malnutrition, specifically calcium and vitamin D nutrition status which is evident from the high incidence of crippling deformities in poor residents from endemic fluorosis zones.^[6,9–11] In addition, exposure to fluoride is also likely to be higher in lower socioeconomic strata (SES) due to lack of access to bottled water (an alternative source of drinking water with permissible fluoride concentration) that serves as a prophylactic measure against fluorosis.

Adequate calcium intake along with sufficient Vitamin D enhances absorption and retention of calcium and thus may modify fluoride absorption in the gut, consequently, affecting the hard tissues (bone and teeth). One of the factors controlling the severity of fluoride toxicity is continuity and duration of exposure to fluoride and the calcium and Vitamin D nutrition status. Research also suggests that adequate calcium nutrition acts as an antagonist which effectively counteracts the toxic effects of fluoride on teeth. Moreover, research on reversal of fluorosis shows that adequate Vitamin D status enhances calcium absorption and retention and thus directly affects the absorption of fluoride ions.^[6,9,12]

Adolescence is a period where 40% of the bone mass is accrued.^[13,14] Thus, it is a critical phase, wherein increased exposure to high fluoride with low dietary calcium intake and decreased serum 25 hydroxyvitamin D₃ (25OHD) concentration may have an impact on the bone and teeth mineralization. Therefore, the aim of this study was to examine: (a) the prevalence and association of

dental and skeletal fluorosis in adolescents from different socioeconomic strata (SES) in an area endemic for fluorosis and (b) the association of dental and skeletal fluorosis with dietary calcium intake and serum 25OHD concentrations.

MATERIALS AND METHODS

A cross-sectional study in apparently healthy adolescents ($n = 90$) aged 10–13.9 years, belonging to upper, middle, and lower SES from a semi-urban region, Patan, Gujarat, Western India, was conducted from January 2012 to March 2014.

Study subjects

A total of six schools, four private schools catering to adolescents from upper and middle SES and two municipal schools catering to adolescents from lower SES were approached. All the schools provided consent for conducting the study. Out of these six schools, two private schools and one municipal school were selected randomly. Stratification of adolescents into upper, middle, and lower SES was performed according to the modified Kuppuswamy's socioeconomic status scale^[15] that takes into account the education, occupation, and family income. It is a composite score of education, occupation, and monthly income of the family that yields a minimum score of 3 and maximum score of 29. Scores ranging from 5–10 were classified as lower SES, between 11 and 15 as middle SES, and above 16 as upper SES as per Kuppuswamy's SES classification. From the selected schools, adolescents and parents willing to take part in the study were interviewed and filled up a screening questionnaire. The adolescents also underwent a clinical examination at the baseline, so as to ensure that they did not have any major illness in the past that may affect their health status. Inclusion criteria comprised apparently healthy adolescents in the age group of 10–14 years. Participants with any chronic ailment or congenital diseases or any major surgery or taking any medications including those which may affect the calcium and Vitamin D metabolism were excluded from the study. Out of those who satisfied the inclusion criteria, a total of 90 adolescents (stratified by gender; 45 girls and 45 boys) were randomly selected from two private schools (upper SES, $n = 30$ and middle SES, $n = 30$) and one municipal school (lower SES, $n = 30$) of Patan. The selection of participants was done using computerized random number generation.

Ethical approval and consent

The purpose of the study and its significance were explained to the principal, administrative authorities, and teachers of the schools along with adolescents and their parents. An informed written consent was obtained from the school

authorities and parents. Ethical approval was granted by the Ethics Committee of Gujarat Medical Education and Research Society General Hospital, Gandhinagar, Gujarat (ECR/535/Inst/GJ/2014). All procedures performed in the study were in accordance with the ethical standards of the Ethical Committee and with the Helsinki Declaration of 1975 (revised in 2000) and its later amendments or comparable ethical standards.

Anthropometry

Height and weight were measured in light clothing without shoes. Standing height was measured to the nearest 1 mm, using a stadiometer (Leicester Height meter, Child growth Foundation, UK, range 60–207 cm). Weight to the nearest of 0.1 Kg was measured (SC240 MA, Tanita, India). Height, weight, and body mass index (BMI) Z-scores were computed using ethnic-specific data.^[16]

Dietary calcium intake

Diet was recorded through a 24 h diet recall taken for three nonconsecutive days (2 weekdays and a Sunday). Nutrient intakes as well as dietary calcium intake were calculated using a cooked and raw food database (C-diet version 2.1, 2013, Xenios Technologies Pvt. Ltd.).^[17-19]

Physical activity

Physical activity assessment was performed by Quantification de L'Activite Physique en Altitude Chez les Enfants questionnaire.^[20]

Dental and skeletal fluorosis

Dental fluorosis was graded as mild (white opacities and/or faint yellow lines, Grade 1), moderate (brown stains, Grade 2), and severe (pitting, chipped off edges, brown plaques, corrosion, and falling of teeth, Grade 3 and 4) by a qualified dentist familiar with fluorosis.^[6] Radiographs of the right hand and wrists were taken to evaluate the development of skeletal fluorosis clinically. The radiographs were examined by two radiologists who were blinded to the study participants and were graded as mild (osteosclerosis only), moderate (periosteal bone formation, calcifications of interosseous membrane, ligaments, muscular attachments, capsules, and tendons), and severe (associated metabolic bone disease [rickets neo-osseousmalacia, osteoporosis, neo-osseous-porosis, and secondary hyperparathyroidism], exostoses, and osteophytosis).^[21]

Biochemical estimation

Serum 25OHD concentrations were measured by chemiluminescent microparticle immunoassay (Abbott, Architect, India; Coefficient of variance 3.7% ± 0.7%) and serum parathyroid hormone (PTH) concentrations were

measured by chemiluminescent immunoassay (Siemens, India; coefficient of variance 3%).

Statistical analysis

Descriptive characteristics (mean and standard deviations [SDs]) were calculated for anthropometric measures, serum 25OHD concentration, serum PTH concentration, calcium, phosphorous, protein, and energy intakes for the study population after confirming normality of data. One way ANOVA, cross-tabulation, and generalized linear model analysis (for relationships between occurrence of fluorosis, SES, serum 25OHD concentration, and calcium intake) was used for analysis. All analyses were performed using SPSS version 18 and the statistical significance (PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc.) level was set at $P < 0.05$.

RESULTS

A total of ninety adolescents (45 boys) aged 10–13.9 years with a mean age of 11.9 ± 1.1 years were studied. All the study participants were permanent residents of Patan by birth and received their drinking water from bore wells (a well, constructed by boring a vertical hole, to extract ground water). We enquired and recorded the source of water in adolescents from all SES, wherein those having access to water having recommended fluoride concentration was referred as bottled water. We found that among our study population, 79% of adolescents from upper SES, 50% from middle SES, and none from lower SES had access to bottled water.^[22,23]

Table 1 summarizes the anthropometric measures, biochemistry, and dietary calcium intake (mean ± SD) for the study population as differentiated by their SES. Mean age did not differ significantly among the SES. Adolescents belonging to upper SES were tallest (height for age Z-score [HAZ] = -0.1 ± 0.7) and heaviest (weight for age Z-score [WAZ] = -0.1 ± 0.9), followed by their counterparts in middle SES (HAZ = -0.4 ± 0.9 , WAZ = -1.1 ± 0.9) and lower SES (HAZ = -1.1 ± 1 , WAZ = -1.5 ± 1); though the differences in HAZ between middle and upper SES and the differences in WAZ between lower and middle SES were not statistically significant. The mean hours of outdoor (during daylight) moderate to vigorous physical activity for low, middle, and upper SES adolescents were 4.3 ± 1.5 , 2.6 ± 0.6 , and 1.9 ± 0.5 h, respectively ($P < 0.05$).

There were no differences in the general characteristics (as judged by HAZ, WAZ, and BAZ) between boys and girls in the study population; hence, the data were pooled. Figure 1 illustrates that dental fluorosis was predominantly seen in adolescents from the lower SES, 17% had both

Table 1: Descriptive characteristics of adolescents belonging to lower, middle, and upper socioeconomic strata

Variables	Lower SES (n=30)	Middle SES (n=30)	Upper SES (n=30)	All (n=90)
Age (years)	12±1.1	11.7±0.9	12±1.2	11.9±1.1
Height (cm)	140.9±9.8 [‡]	144.1±6.2	147.4±8.2	144.1±8.5
Weight (kg)	30.3±8.2 [‡]	31±5.3 [‡]	40.2±9.3	33.8±8.9
BMI (kg/m ²)	15±2.2 [‡]	14.9±2.2 [‡]	18.4±3.4	16.1±3.1
HAZ	-1.1±1* [‡]	-0.4±0.9	-0.1±0.7	-0.5±1
WAZ	-1.5±1 [‡]	-1.1±0.9 [‡]	-0.1±0.9	-0.9±1.1
BAZ	-1.2±0.8 [‡]	-1.2±0.9 [‡]	-0.1±1.1	-0.9±1.1
Serum 25OHD (ng/ml)	26.3±4.9* [‡]	23.4±4.7 [‡]	18.6±4	22.8±5.5
PTH (pg/ml)	33.2±13.4 [‡]	25.5±19.8	23.3±13.9	27.3±16.3
Ionized calcium (mmol/L)	1.17±0.03* [‡]	1.20±0.04	1.21±0.03	1.19±0.04
Serum calcium (mg/dl)	9.2±0.2* [‡]	9.5±0.3	9.6±0.2	9.4±0.3
Dietary calcium intake (mg/day)	441.2±227.6 [‡]	484.3±160.9 [‡]	749.2±245.4	558.2±252.4
Moderate to vigorous activity (h/day)	4.3±1.5* [‡]	2.6±0.6 [‡]	1.9±0.5	2.9±1.4

All values are mean±SD. *Mean values are significantly different between lower SES and middle SES ($P < 0.05$), [‡]Mean values are significantly different between lower SES and upper SES ($P < 0.05$), [‡]Mean values are significantly different between middle SES and upper SES ($P < 0.05$). HAZ: Height for age Z score, WAZ: Weight for age Z score; BAZ: BMI for age Z score, BMI: Body mass index, SES: Socioeconomic strata, SD: Standard deviation, PTH: Parathyroid hormone, 25OHD: 25 hydroxyvitamin D3

dental and radiological features, whereas 73% had dental fluorosis (33% suffered from mild whereas 40% suffered from moderate to severe dental fluorosis). Only 10% of the lower SES adolescents were not affected by fluorosis. Thirty-three percent from upper SES and 55% from middle SES suffered from dental fluorosis whereas only 7% had both dental and radiological features ($P < 0.01$). However, no skeletal deformities were observed in any of the study adolescents [Figure 1]. Overall, irrespective of the SES, 27% adolescents suffered from mild dental fluorosis, 27% suffered from moderate to severe dental fluorosis, 10% suffered from both dental and skeletal fluorosis whereas 36% adolescents were normal ($P < 0.001$).

Mean serum 25OHD concentrations were 26.3 ± 4.9 , 23.4 ± 4.7 , and 18.6 ± 4 ng/ml for lower, middle, and upper SES, respectively ($P < 0.05$). Lower SES adolescents had significantly higher PTH concentrations (33.2 ± 13.4) than upper SES adolescents (23.3 ± 13.9) ($P < 0.05$). Sixty-three percent adolescents from upper SES, 27% from middle SES, and 7% adolescents from lower SES were deficient in serum 25OHD (< 20 ng/ml)^[24] ($P < 0.001$). Ten percent of the lower SES adolescents and 3% adolescents each from middle and upper SES had intact serum PTH concentrations above the reference range (9–55 pg/ml)^[25] ($P = 0.328$). Mean dietary calcium for lower, middle, and upper SES was 441.2 ± 227.6 , 484.3 ± 160.9 , and 749.2 ± 245.4 mg/day, respectively ($P < 0.001$). Compared to the recommended dietary allowance (RDA) for calcium (800 mg/day), lower and middle SES had calcium intake below the RDA whereas upper SES had calcium intake close to the RDA.^[26]

To investigate the impact of SES, serum 25OHD concentrations, and calcium intake on the occurrence of fluorosis in adolescents, generalized linear model analysis was carried out. The results showed that SES had a significant

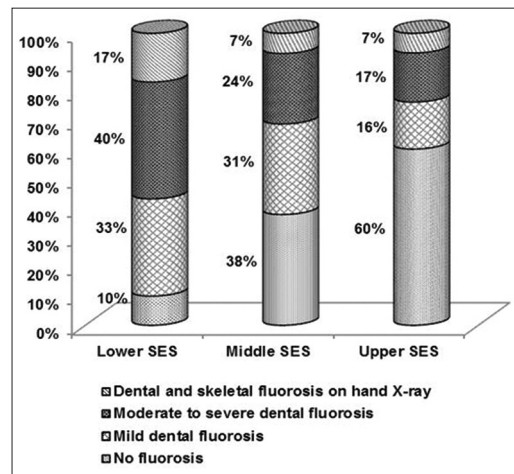


Figure 1: Prevalence (%) of fluorosis in lower, middle, and upper socioeconomic strata (SES) ($P < 0.01$)

association with dental and skeletal fluorosis (exponential $\beta = 2.5$, $P = 0.011$). For lower SES, the risk of occurrence of fluorosis was highest, followed by middle and upper SES. As compared to upper SES, middle SES adolescents were at 1.3 times whereas lower SES adolescents were at 2.5 times higher risk. Serum 25OHD concentrations ($P = 0.937$) and dietary calcium intake ($P = 0.825$) did not show a significant association with dental and skeletal fluorosis as judged by generalized linear model analysis.

DISCUSSION

We observed that the prevalence of fluorosis varied among adolescents from different SES. The incidence of dental and skeletal fluorosis was significantly associated with SES, and nearly three-fourth of the lower SES adolescents suffered from mild and moderate to severe dental fluorosis, whereas one-fourth showed both dental and radiological signs. Three-fourth of the upper SES, one-half of the

middle SES, and none of the lower SES adolescents had access to bottled water. Having access to bottled water reduced the exposure to fluoride, thus, low SES working through poor accessibility to bottled water, was possibly associated with an increased risk of fluorosis. Another possible mechanism why low SES adolescents were the most affected by fluorosis is the lack of education and awareness of fluorosis.

Dietary calcium intake and Vitamin D status are significant factors responsible for the severity of fluorosis as the deficiency of these nutrients aggravate fluorosis through increased absorption of fluoride in the hard tissues.^[9,12,21,27] We thus examined the association of fluorosis with dietary calcium intake and serum 25OHD concentrations in our study adolescents. We found that there was no significant association of fluorosis with serum 25OHD concentrations and dietary calcium intake. The probable reasons for such a result may be that though dietary calcium intake was below RDA (55% and 60%, respectively) in lower and middle SES; however, it was still above 50% RDA; moreover, lower and middle SES adolescents also had serum 25OHD concentrations above the deficiency range^[24] (26.3 ± 4.9 and 23.4 ± 4.7 ng/ml, respectively, for lower and middle SES). Thus, relatively adequate serum 25OHD concentrations may have been responsible for the increased absorption of dietary calcium so that the effect of high fluoride was partially masked; this was probably the reason for no skeletal abnormalities in our study participants. Although other studies have obtained a negative association between milk consumption and dental fluorosis, in line with our observations, they have also reported that as the fluoride concentration increases this association is lost.^[27]

Our study also revealed that lower SES adolescents were the most affected with the highest prevalence of dental (73%) and skeletal fluorosis (17%). Similar observations have also been reported by others.^[28,29] Studies conducted in endemic fluoride regions, targeting poor economic status population have reported 92%–100% prevalence of dental fluorosis in rural children and adolescents.^[9,27] Our results clearly indicate that with relatively sufficient serum 25OHD concentration and dietary calcium intake, the effect of fluoride can be counteracted, thus reducing the incidence of severe skeletal fluorosis. In line with our results, Khadare *et al.* compared children with and without skeletal deformities in high fluoride villages and found that children with skeletal deformities had significantly low serum 25OHD concentration and dietary calcium intake than children without skeletal deformities.^[30]

The strength of our study is that we have examined the association of SES, working through poor access to safe

drinking together with calcium and vitamin D status on the occurrence of fluorosis. Our limitations include the fact that we have performed X-ray of the hand and forearm; however, the lower limbs could not be radiographed. Nevertheless, none of the adolescents showed any skeletal deformities of the limbs or spine. Furthermore, our observations target a common but important problem that needs the application of appropriate public health strategies; nevertheless, adequately powerful intervention programs are required to confirm the findings that we have observed from a small observational study.

CONCLUSION

Fluorosis was more common in lower SES adolescents, probably due to the lack of access to bottled water. Relatively, adequate calcium intake and serum 25OHD concentrations may have increased the efficiency of dietary calcium absorption, thus preventing severe fluorosis. There is an urgent need to provide safe drinking water in areas endemic for fluorosis as also to improve the calcium and vitamin D status of these adolescents.

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Conflicts of interest

There are no conflicts of interest.

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