STUDY PROTOCOL

Prevalence of Dental Fluorosis among 6-18-Year-Olds in Vidarbha, Maharashtra, and Its Association with Geological and Geochemical Fluoride Levels: A Cross-Sectional Study

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ABSTRACT

Background: Endemic Fluorosis is a longstanding public health problem caused by excess fluoride intake through drinking water or food products grown in soil with high fluoride content. Many areas in Maharashtra state, particularly the Vidarbha region are affected by fluorosis. The aim was to determine the prevalence of Dental Fluorosis in children of the age group of 6-18 years and corelate it with the fluoride levels in water, soil and foodgrains.

Methods and Materials: A cross-sectional study will be conducted in randomly selected villages from Vidarbha region using multistage sampling. A total sample of 1230 children aged 6-18 years will be obtained. Dean`s index will be utilized for determining the prevalence of Dental Fluorosis. Fluoride levels of drinking water, soil and foodgrains will be analyzed.

Results: The salient feature of the study is the fluoride levels in the drinking water and locally grown staple foodgrains like rice, jowar and wheat etc. This will generate evidence that corroborates with the fluorosis levels observed in the selected study region.

Conclusion: The study will be a landmark in nearly a decade providing a comparative account of Dental Fluorosis in villages with high-to-moderate endemicity and co-relate clinical manifestations with fluoride levels in water, soil and foodgrains.

Keywords: Fluoride, Dental Fluorosis, Children, Foodgrains, Water, Geological, Geochemical

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INTRODUCTION

Endemic Fluorosis is a one of the chronic public health problems caused by excess intake of fluoride through drinking water, consumption of food products grown in soil with high fluoride content and exposure to industrial emissions over a long period of time.[1] It is represented by Dental Fluorosis that is defined as a condition that occurs when too much fluoride is consumed while teeth are developing, resulting in discoloration or other changes to the dental enamel. A miscellaneous source of exposure could be through fluoride supplements.[2] Industrial emissions can contribute to dental fluorosis by releasing fluoride into the air, soil, and water, which can then contaminate food, water, and vegetation thereby integrating into the food chain and causing accumulation over years leading to cumulative effects. Similarly, supplements in the form of fluoride content of the toothpastes causes a similar effect of slow accumulation if too much of fluoride is deposited in the developing teeth.[2]

Fluoride in low quantity is useful to humans but high quantity leads to several disorders and also it affects each and every part of the human body.[3] Excessive intake of fluoride leads to toxicity, predominantly affecting the teeth and skeletal system. There can secondary involvement of nervous system in advanced stages causing crippling fluorosis.[4]

The spectrum of fluorosis is dental fluorosis, skeletal fluorosis and non-skeletal fluorosis. Advanced stages of dental and skeletal fluorosis can lead to permanent, irreversible changes that are detrimental to both individuals and the broader community. This can amount to huge losses on growth, development & economy of the country.[5-7]

Global Burden: Fluorosis can be seen distributed around the world, being endemic in at least 25 countries. The fluoride belts are reported in South African countries stretching from Syria to Kenya including Algeria, Egypt, Jordan, Sudan and Libya.[8,9] The other fluoride belt stretches from Middle east through India to China along with Turkey, Iraq, Iran, Afghanistan and northern Thailand. There are similar belts in the America and Japan.[10]

National Burden: In India 230 districts have reported high levels of Fluoride among 20 states (after bifurcation of Andhra Pradesh in 2014). The population at risk for fluorosis is 11.7 million according to the 2014 estimates. There is a scarcity of recent data on the prevalence of fluorosis at the population level as there were no community-based studies conducted in the last decade. The worst hit states are "Andhra Pradesh, Rajasthan and Gujarat" followed by "Maharashtra, Punjab, Madhya Pradesh and Haryana. States like Tamil Nadu, West Bengal, Uttar Pradesh, Bihar and Assam" are mildly affected.[11-13]

In parts of Gujarat and Uttar Pradesh cause of fluorosis is due to industrialization whereas, water is the common source of fluorine in rest of the India. The central belt comprising of states from east to west including Gujarat, Maharashtra, Rajasthan, Orissa, and West Bengal are mainly affected by high fluoride levels in the drinking water.[11,12] This is the chief contributing factor for the high level of fluorine in the Vidarbha region along with the rapidly expanding development projects like construction activities, groundwater abstraction as well as heavy usage of fertilizers containing fluorides that leech into the ground contaminating the sources of drinking water. The desirable limit of fluoride as per "Bureau of Indian Standards" (BIS) is 1ppm (parts per million or 1 mg per liter). The World Health Organization (WHO) recommends optimal fluoride levels of 0.5-0.8 ppm.[14]

Burden in Maharashtra: In Maharashtra state many parts are affected by fluorosis showing moderate endemicity. The level of endemicity is decided upon by the extent of affliction of the teeth surface as per the commonly used classifications for Dental Fluorosis which states that moderate level of endemicity is represented by uniform brown discolouration of the tooth surface affecting majority of the dental enamel in most of the teeth. This scenario is commonly observed in the selected study areas. The reason for fluorosis in these areas is consumption of water with fluoride levels of 0.8-10 ppm.[15] There is dearth of studies conducted at regional levels which could show exact burden of dental fluorosis though lot have been conducted in different districts and villages.[16-18]

Rationale for the proposed study: There are very few studies conducted on the prevalence of dental fluorosis in the past decade. Also, studies providing estimates for the soil and water levels of fluoride are few and sparse. Moreover, studies that co-relate clinical manifestations with fluoride levels in water, soil and foodstuff have not been conducted in the region (Vidarbha region of Maharashtra), despite being a high-risk area affected by fluorosis with moderate endemicity. The additional analysis of the water, food and soil samples will yield data that can be corroborated with the clinical picture observed in the region which will be useful for establishing epidemiological corelations with the water, soil and food samples tested in the study. Information on the topographical characteristics with regards to the water and soil levels of fluorides is also not available. In this context, the present study was designed with the following objectives:

Objectives:

Primary Objective of the research is to study the prevalence of Dental Fluorosis among children aged 6-18 years in selected districts of Vidarbha region of Maharashtra.

Secondary Objectives includ assessment of fluoride levels in water, soil and foodgrains/ vegetables grown and consumed locally in the region; and also to determine the association of the fluoride levels in water, soil and staple foodgrains like rice, wheat and

jowar that are grown locally with dental fluorosis in children aged 6-18 years in selected districts of Vidarbha region of Maharashtra.

Hypotheses for secondary objectives:

Null Hypothesis: There is no statistically significant association of the fluoride levels in water, soil and foodgrains with Dental Fluorosis in children aged 6-18 years in selected districts of Vidarbha region of Maharashtra.

Alternative Hypothesis: There is a statistically significant association of the fluoride levels in water, soil and foodgrains with Dental Fluorosis in children aged 6-18 years in selected districts of Vidarbha region of Maharashtra.

METHODOLOGY

An analytical cross-sectional study will be conducted after the approval from institutional ethics committee with the following methodology.

Study Design: Analytical cross-sectional study. The study design is chosen to study the prevalence of Dental Fluorosis which will be followed by intra group comparison to determine the association of prevalence of Dental Fluorosis with the levels of fluoride in water, soil and food grains.

Study Setting: The study will be conducted in 18 villages from three selected districts from the Vidarbha region of Maharashtra state. The study will be conducted among **c**hildren aged 6-18 years residing in the study area. Total study Duration will be one year.

Sample size: According to the study by Jakati et al. 2017,[19] conducted at the Waghdhara village near Nagpur district, dental fluorosis was observed among 80.0% of the children aged 11-18 years. Therefore, considering a prevalence of 80.0%, with 5% absolute precision and 95% confidence intervals, the sample size calculated using Open Epi software was obtained to be 369 per district. Considering 10% non-response rate, the sample size was 410 per district. Thus, a total sample of 1230 will be recruited in the study.

Sampling Technique: Multistage sampling will be utilized to select the participants from the rural areas of the selected districts. The Probability Proportional to Size technique will be used to select the required number or rural (villages) regions from each district in order to meet the sample size. Five villages will be selected by simple random sampling method from each district. The number of children aged 6 – 18 years will be obtained from the gram-panchayat and listed accordingly. The children will be included in the study proportionately by simple random sampling from each village to achieve the sample size for the particular district. If the selected child is not present or not willing to participate, then next child in the list will be approached and included in the study.

Inclusion criteria: All children aged 6-18 years who are having permanent teeth with at least more than 50% of the crown erupted and no cavity filling on facial surface. Permanent tooth is a tooth in a mammal that replaces a temporary milk tooth and lasts for most of the mammal's life. Children whose parents are willing to give consent will be included in the study.

Exclusion criteria: Children with developmental defects of teeth or the absence of permanent teeth will be excluded from the study.

Statistical Analysis: The data will be entered in MS excel. Descriptive statistics like percentage, means and standard deviations will be calculated wherever required. ANOVA and post-Hoc Tukey test will be applied to compare the difference between mean values of fluoride of different groups. This will be followed by determining the association of the fluoride content of grain with water and soil will be seen by applying Linear regression method. P value of <0.05 will be considered significant. The Epi-info software version 7.2.2 will be used for analysis. The software is developed by CDC Atlanta and is recommended for community-based research.

Methodology: After obtaining the ethical clearance from the Institutional Ethics Committee, a cross-sectional study will be conducted in three selected districts among children in the age group of 6-18 years. In Vidarbha region, of the 11 districts, 3 districts are moderately affected by Fluorosis, from which 2 districts will be selected by simple random sampling for the study and a third district will be selected from those which are mildly affected by fluorosis as a control comparison group. The rural regions will be selected based on probability proportionate to size and the sample of 1230 children in the age group of 6-18 years will be recruited by simple random sampling from the selected areas. The study timeline is shown in table 2.

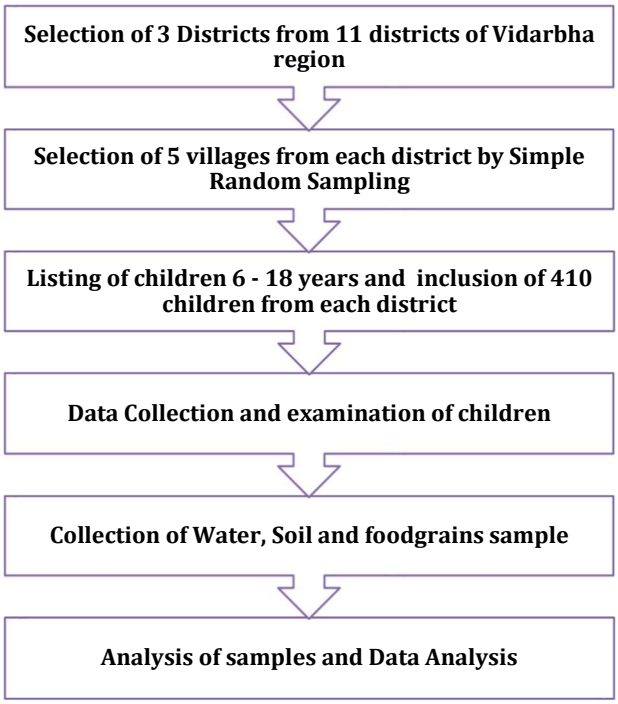
Table 1: The Dean's Index Score: Modified Criteria for assessing the grading of Dental fluorosis

Score	Weight	Description
Normal	0	No white spots or flecks
Questionable	0.5	few white flecks to occasional white spots
Very mild	1.0	Small, opaque paper white areas < 25% of the tooth surface
Mild	2.0	Small, opaque paper white areas 25-50 % of the tooth surface
Moderate	3.0	Brown stain is a disfiguring feature.
Severe	4.0	Discrete or confluent pitting, Change in anatomical form of tooth.

Study procedures: The oral examination of children after taking informed consent from the parents for Dental Fluorosis will be done by a trained Dentist. The sociodemographic data, dietary data, sources of drinking water and information on oral hygiene practices will be collected using a pretested questionnaire by interview technique by the study staff. The study staff will be trained for data collection by the investigators. Survey of fluorosis in the community including school children will be conducted as per surveillance guidelines for assessment and diagnosis of fluorosis cases the age group of 6-18 years at the community level. The diagnosis of fluorosis is done by using DEAN'S Fluorosis index: Modified Criteria will be used to diagnose fluorosis. It is a 6-point ordinal scale with scoring system ranging from 0 (Normal) – 4 (Severe).

Analysis of Fluoride levels in water, soil and food: Proportional samples of soil, water and grains from each area of residence of the children will be collected following standardized method from the fields of the same village which were used by the villagers. One sample of soil, water and grains will be collected from each village. 100 grams of soil sample will be collected from one of the farms in the village and will be stored in plastic bag, labelled appropriately and sent to the laboratory. 100 ml sample of water will be collected from the drinking source of the village by the help of a sterile container and stored in sterile plastic container, labelled and sent to the laboratory for analysis. Similarly, 100 grams of the locally grown grains i.e. rice or wheat will be collected from the local farmer and sent in a plastic bag for analysis. All the samples will be collected in the similar containers, labelled appropriately and sent to the laboratory within a week. SPANDS technique will be used to find Fluoride ion concentration of the samples.[20] The Mean difference of fluoride levels will be utilized to find difference between groups by using ANOVA and Post-Hoc Tukey test. To analyse the association of the fluoride content of grain with water and soil Linear regression method will be applied.

SPADNS Spectrophotometric Method: The zirconium dye reacts with Fluoride ions and a colourless complex along with a dye is formed.[21] The higher the fluoride concentration the lighter will be the colour of dye. Absorbance is measured at 570 nm. Absorbance values for known standards will be used to formulate a calibration curve. This will give the fluoride values for the samples.[22]



Flow Chart 1: Sequential Plan of study

Table 2: Project timeline for one year

Gantt Chart – Timeline for project

Activities	Q1*	Q2	Q3	Q4
Protocol Development				
Development of study tools				
University Ethical Approval				
Data Collection at 3 Districts				
Data entry and data cleaning				
Sample Analysis and Data Analysis				
Writing the research article				
Publication				

*Q: Quarter (3 months duration)

RESULTS

There is a dearth of studies regarding dental fluorosis since last decade especially in rural areas though the problem is persistent in many districts of Vidarbha. The study results will give a reliable and accurate estimate for the overall prevalence and grade the severity of Dental Fluorosis among children in the age group of 6-18 years. The association of dental fluorosis with fluoride levels in the drinking water sources, soil and foodgrains will be studied. The data on the fluoride levels in the drinking water from the study sites as well as the levels of fluoride in the locally grown staple foodgrains like rice, jowar and wheat etc. will generate evidence that corroborates

with the fluorosis levels observed in the selected study region.

Discussion

The study will be instrumental in providing the prevalence of dental fluorosis in children in the age group of 6-18 years, which has been seldom studied and will also give an insight into the probable sources of the fluoride present in the community. The target age group is unique to the present study as it will reflect the changes related to fluoride exposure in both sets of milk and permanent teeth in the respective ages.[7-9] Studies conducted in the region in the last decade on dental fluorosis are sparse and localized to a district or limited to few villages only.[9,12] The strategic selection of the Vidarbha region as well as the districts within based on the severity or endemicity of dental fluorosis, reflects the availability of a comparison group from moderately endemic region that will serve as a comparator for both the prevalence determinants and the water soil and food levels of fluoride in the high, moderate and low endemic areas. Previous studies are conducted in lower age groups and very few studies conducted in the past few decades have corelated the evidence with the loco-regional sources of fluoride with the levels of fluorosis.[12,15] This is particularly observed with water levels in the previous studies but rarely conducted for soil and the locally grown food grains that form the major part of the staple diet of the residents of these villages.[13,17] Thereby providing the much needed evidence for the formulation of potential strategies for its prevention through the development of targeted interventions.

Conclusion

The study will be a landmark in nearly a decade and half to provide a comparative account of dental fluorosis in villages from 3 districts of Vidarbha that have high and moderate endemicity and co-relate clinical manifestations with fluoride levels in water, soil and foodgrains. This will help in formulating policies if required for curbing the problem.

Authors' Contributions

SH developed the concept and designed the study protocol along with writing the initial draft of the manuscript and refining it to the current level. YP helped with the writing of the protocol and the manuscript and approved the present version. AS helped with the development of the protocol and approved the present version of the manuscript.

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