INTRODUCTION

Fluorine is one of the 14 physiologically essential elements required for normal growth and development but does not exist in nature in its elemental state. The ionic form fluoride is found abundantly in a wide variety of minerals, including fluorspar and rock phosphate. Due to the universal presence of fluorides in the earth’s crust, all water sources contain fluorides in varying concentrations.

Fluoride has been termed as the double-edged sword. When ingested in optimum concentration, fluoride shows preventive action against dental caries. But when ingested in excess, it may lead to dental or skeletal fluorosis.

Consumption of high levels of fluorides from several sources can be detected by means of certain biomarkers. Biomarkers do not diagnose diseases but are an indicator of the disease or an altered physiological state. Plasma, bone, teeth, urine, saliva, dental plaque, plaque fluid, hair, and nails serve as exposure biomarkers for fluorosis, while genetic factors, acid-base disturbances, renal disturbances, bone growth, and nutritional state are the susceptibility biomarkers. Reduction in the activity and severity of the dental caries, dental fluorosis, and skeletal fluorosis may be considered as the biomarkers of effect.[2]

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The contemporary biomarkers for fluoride have been urine, plasma, and salivary fluoride content. However, these do not measure the chronic fluoride exposure. Moreover, urinary fluoride excretions and concentrations are variable because of variations in urinary flow and pH. Hence, hair and nails are considered as the recent biomarkers and reflect the average plasma fluoride concentrations over time.\[3\]

In India, endemic fluorosis is considered to be a major public health problem. According to the available literature, more than 15 states in India are endemic for fluorosis, and more than 60 million people in India suffer from fluorosis, either dental or skeletal.\[11\]

Analyzing of fluoride intake through hair samples is not a routine practice but is gaining importance, especially when there are instances of environmental exposure to excess fluoride.\[4\]

Excess fluoride consumption hence shows effects on both teeth, as well as chronic deposition in hair. Thus, the present study was conducted with the aim to evaluate scalp hair as biomarker of chronic fluoride exposure among the fluoride endemic and low fluoride areas and to correlate fluoride content in hair with the levels of dental fluorosis.

**METHODOLOGY**

This was a cross-sectional study, which involved the collection of scalp hair samples from Ajod, a low fluoride area (fluoride content in drinking water – 0.11 ppm) and Karsan, a fluoride endemic area (fluoride content in drinking water – 3.43 ppm) from Vadodara district.\[5\]

Included in the study were the individuals in the age group of 35–60 years, who gave their informed written consent for the study and those who were living in the given locality since birth. Individuals living outside the study area, and those who did not agree to participate were excluded from the study.

The sample size was obtained using data from the previous similar study conducted by Parimi et al.\[6\] The minimum number of participants required to get probability of average hair score of 0.9 of one group higher than second at 1% risk and 90% power was 36. Hence, 18 participants were selected from each village to fulfill the minimum sample size. Systematic random sampling was carried out to enroll the participants who met the inclusion criteria.

The Modified Thylstrup Fejerskov Index (TFI)\[7\] [Table 1] was used to record the severity of dental fluorosis in the study participants.

The participants were asked to complete a questionnaire asking for general data: age, gender, water supply, and place of residence since birth and oral hygienic practices.

Samples of hair of minimal length 2–3 cm were taken from the occipital region using a pair of scissors and collected in a self-lock clear plastic polybag. After every use, the excess hair was brushed off the scissor blades using a small brush. The pair of scissors was then cleaned using sanitizer and cotton ball before collecting hair sample from next participant.

Following procedure was carried out for analysis of the hair samples:

1. Samples of hair were placed in a fritted glass filter and redistilled water. After drying, 100 mg aliquots

| Table 1: Modified Thylstrup Fejerskov Index |
|-------------------------------------------|
| Score | Criteria (Fejerskov et al., 1988) |
| TF score 0 | The normal translucency of the glossy creamy white enamel remains after wiping and drying of the surface |
| TF score 1 | Thin white opaque lines are seen running across the tooth surface. Such lines are found on all parts of the surface. The lines correspond to the position of the perikymata. In some cases, a slight “snow-capping” of cusps/incisal edges may also be seen |
| TF score 2 | The opaque white lines are more pronounced and frequently merge to form small cloudy areas scattered over the whole surface. “Snow-capping” of incisal edges and cusp tips is common |
| TF score 3 | The white lines merge, and cloudy areas of opacity can be seen spread over many parts of the tooth surface. In between the cloudy areas, white lines can also be seen |
| TF score 4 | The entire surface exhibits a marked opacity or appears chalky white. Parts of the surface exposed to attrition or wear may appear to be less affected |
| TF score 5 | The entire surface is opaque, and there are round pits (focal loss of outermost enamel) that are <2 mm in diameter |
| TF score 6 | There is merging of the small pits into bands, with a vertical height of <2mm. In this class are included also the surfaces where the cuspal rim of facial enamel has been chipped off, and the vertical dimension of the resulting damage is <2 mm |
| TF score 7 | There is a loss of outermost enamel in irregular areas, and less than half the surface is involved. The remaining intact enamel is opaque |
| TF score 8 | The loss of the outermost enamel involves more than half the enamel. The remaining intact enamel is opaque |
| TF score 9 | The loss of the major part of the outer enamel results in a change of the anatomic shape of the surface/tooth. The cervical rim of opaque enamel is often noted |

TF – Thylstrup Fejerskov
were placed into test tubes, treated with concentrated sodium hydroxide solution and heated in a boiling water bath until complete solution (hair 60 min). Cooled and neutralized with 1 M HCl acid and the sample volumes were made up with water to 4 ml and diluted with equal volumes of total ionic strength adjustment buffer. Fluoride concentrations were measured by a fluoride ion specific electrode and an Ag/AgCl reference electrode with a double jacket.[6]

The collected data was entered in Microsoft (2010) spreadsheet. Descriptive statistics was used to calculate the frequency and percentage of participants suffering from different grades of dental fluorosis in the respective low fluoride and fluoride endemic areas as interpreted from the modified TFI. Mann–Whitney U-test was used to compare the mean fluoride content in the scalp hair between the two groups. Chi-square test of association was used to associate the grade of dental fluorosis with the fluoride content of scalp hair for the two groups.

RESULTS

There were a total of 36 individuals who participated in the present study. The distribution of the participants was equal in the two areas chosen for the study. There were 18 participants from Karsan, which was identified as the fluoride endemic area in Vadodara district and 18 from Ajod, identified as a low fluoride area in Vadodara district. Figures 1 and 2 show the gender-wise and age-wise distribution of the participants, respectively. The TFI scores of the participants are illustrated in Figure 3.

The mean fluoride content in hair samples of participants from Karsan was found to be 3.40 ppm (±1.043). Similarly, the mean fluoride content in hair samples of participants from Ajod was found to be 0.35 ppm (±0.063). The difference in means between the fluoride content in hair was also found to be statistically highly significant using the independent t-test [Table 2].

When plotted on a graph, the TFI scores and the scalp hair fluoride content showed a positive association [Figure 4].

DISCUSSION

Fluorine is the most abundant element in nature and is essential for the normal mineralization of bone and formation of dental enamel. Yet, fluorosis remains a very important problem in more than 24 countries in the world, including India, which lies in the geographical fluoride belt that extends from Turkey to China and Japan through Iraq, Iran and Afghanistan.

The World Health Organization (WHO) has set the upper limit of fluoride concentration in drinking water at 1.5 mg/dl. The Bureau of Indian Standards has therefore laid down Indian Standards as 1.0 mg/dl as maximum permissible limit of fluoride with further remarks as “lesser the better.” Intake of fluoride higher than the optimal level is the main reason for dental and skeletal fluorosis. The available data suggest that 15 states in India are endemic for fluorosis (fluoride level in drinking water >1.5 mg/l), and about 62 million people in India suffer from dental, skeletal, and nonskeletal fluorosis. Out of these, 6 million are children below the age of 14 years.

Table 2: Comparison of TFI Score and Fluoride Content in hair between Fluoride endemic and low fluoride areas

| Group          | n  | Mean±SD  | t value | Degree of freedom | P         |
|----------------|----|----------|---------|-------------------|-----------|
| TFI score      |    |          |         |                   |           |
| Karsan         | 18 | 3.39±0.979 | 8.639   | 34                | <0.001*   |
| Ajod           | 18 | 0.83±0.786 |         |                   |           |
| Fluoride content in hair (ppm) |    |          |         |                   |           |
| Karsan         | 18 | 3.40±1.043 | 12.370  | 34                | <0.001*   |
| Ajod           | 18 | 0.35±0.063 |         |                   |           |

*Highly significant. TFI – Thylstrup Fejerskov Index; SD – Standard deviation

Figure 1: Gender-wise distribution of participants

Figure 2: Age-wise distribution of participants
considered as the major source of drinking water in most places on earth.\[8\]

Time and again, it has been proven that the levels of fluoride in water are directly proportional to the severity of dental fluorosis. High levels of fluoride in biomaterials such as urine and blood have been proposed as the most reliable indicator of the exposure to fluorides.\[3\]

In the present study, the fluoride content in the hair of participants from endemically fluoridated area was much higher than the participants of low fluoridated area. This finding is consistent with the results of study conducted by Parimi et al.\[6\] Similar results were also found by Czarnowski and Krechniak\[4\] and Kokot and Drzewiecki.\[9\]

The increase in the fluoride content of hair depends on the degree of exposure to fluorides sources, especially water. Trace elements including fluorides, which are of endogenous origin, are absorbed into the blood and subsequently incorporated into the keratin structure of hair during the growth phase. In the present study, cleaning procedure was carried out during the laboratory procedure, to eliminate surface contamination. Hence, only the fluoride of endogenous origin was measured, a major source of which being fluoride content of drinking water.

The inclusion criteria for selection of the participants were that they should be residing in the said villages since birth. Hence, the accumulation of fluoride in hair in the fluoride endemic area was a cumulative process over time, which could be demonstrated by the high fluoride levels in hair of the participants from the fluoride endemic area, as compared to the low fluoride area.

Water is the major source of fluoride intake in humans. Consuming appropriate and relatively small amounts of fluoride by water is generally conceived to have a beneficial effect. However, intake of water excessively rich in fluoride results in pathological changes in teeth, leading to dental fluorosis. This has been clearly demonstrated in the present study. The TFI scores were higher for the participants chosen from Karsan, which is a high fluoride area, as compared to Ajod, which is a low fluoride area. These findings are consistent with several other studies conducted in the past. Indermitte et al.\[10\] studied the risk of dental fluorosis in Estonia on exposure to high fluoride drinking water and found a strong correlation between natural fluoride levels in drinking water and the prevalence of dental fluorosis. Similar positive correlation was also confirmed between fluoride content of well water and dental fluorosis level in a study conducted by Mandinic et al.,\[11\] who studied fluoride in drinking water and dental fluorosis in different Serbian municipalities. Firempong et al.\[12\] who carried out a study to investigate the relationship between fluoride ions in drinking water and the incidence of dental fluorosis in some endemic areas of Bongo district, Ghana, also found positive correlation between the two. Dean's specific index was used to assess dental fluorosis.

In the present study, a positive linear correlation was found between the fluoride levels in hair of the participants and the TFI scores. The results are similar to the results presented by Mandinic et al.\[11\] in their study to assess the relationship between dental fluorosis and fluoride content in hair in school children from fluorotic and nonfluorotic regions in Serbia. Statistically significant correlations were confirmed between levels of fluoride in hair and prevalence of dental fluorosis.\[11\]

This may be because the most common sites where the deposition of endogenous fluoride takes place during the growth phases are the hair and teeth. Thus, exposure to high contents of fluoride from drinking water up to the age of 8 years will lead to dental fluorosis. At the same time, fluoride will also get deposited in the keratin...
of hair, and hair being an indicator of chronic fluoride exposure shows the presence of fluoride when treated chemically.

From the present study, it is realized that the fluoride ion concentrations of Karsan village is above the WHO recommended value of 1.5 ppm for public water supplies. The human body has no mechanism to regulate excess fluoride levels. Thus, when villagers ingest the excess fluoride, it leads to dental fluorosis. This excess fluorosis also gets deposited in scalp hair, which was detected in the present study. Similar theory may be applied to other areas which fall in the fluoride endemic belt. There is therefore a need to drastically reduce the fluoride ions in drinking water for such fluoride endemic communities.

Alkaline metals are loosely bound to hair, while alkaline earth metals are bound at a greater extent, which can be observed during removal of metals from hair in detergents. Metal analysis of hair concerning the process of incorporation of metals can also provide significant clues in the identification of metal toxicity and also the deficiency of essential metals. Since fluoride endemic belts have already been identified throughout the world, fluoride content in hair can also be used to identify geographical location and environmental conditions in forensic investigations. Ecological elements are of vital importance in forensic identification of cases, and hair has played the key evidence since the last century.[13]

Hair analysis should thus play a greater role in routinely measuring the chronic exposure to fluoride.

**CONCLUSION**

The current study found that there is a significant association between the fluoride content in drinking water and fluoride content of scalp hair. We can thus conclude that apart from urine and blood, hair can also contribute as biomaterials for monitoring the chronic fluoride exposure. Urine and blood serve as short term indicators of fluoride exposure. On the other hand, scalp hair is a long-term fluoride exposure indicator. Hair samples can be collected by easy, painless, and noninvasive methods, giving them further advantage over the other biomaterials. Fluoride ion-specific electrodes should be made more easily available and accessible so that the scope of hair samples as biomaterials can be further explored in different places using larger samples.

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**Conflicts of interest**

There are no conflicts of interest.

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