Progression of Artificial Caries in Fluorotic and Nonfluorotic Enamel. An *in vitro* Study

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Background and objectives: *Fluorosis is an important clinical and public health problem in several parts of the world. Although the relationship of fluoride level in drinking water to dental caries and dental fluorosis is known, relationship of fluorosis with the caries is not clear. This study was conducted to evaluate and compare the thickness of enamel and depth of lesion after inducing artificial caries in fluorotic and nonfluorotic teeth.* ***Methods:*** *Study group included 15 fluorosis affected and 15 normal teeth. Artificial caries was induced and teeth were sectioned to 150 microns and observed under polarized light microscope to measure the enamel thickness and depth of lesion in microns.* ***Results:*** *Statistical analyses of the measurements were made using student's unpaired t-test. Thickness of the enamel of nonfluorotic teeth was found to be significantly more when compared with the fluorotic teeth(p-value 0.0404) and depth of lesion was significantly more in fluorotic teeth when compared with the nonfluorotic teeth(p-value 0.0218).* ***Conclusion:*** *Although fluoride is acknowledged as an essential factor in the prevention of dental caries there has to be careful balance in the amount consumed to ensure that fluorosis does not occur.*

Keywords: *Dental fluorosis, Caries prevalence, Hypoplasia, DMFT Scores*
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INTRODUCTION

The decline in caries prevalence observed in many developed countries has been attributed to the judicious use of fluorides in different forms. Use of systemic fluorides in children results in ingestion and absorption of fluoride into the blood stream. The mineralization of teeth under formation may be affected either positively making them caries resistant or negatively resulting in fluorosis depending on the fluoride levels.[1,2] Fluorosis affects the forming enamel to cause porosity of the enamel. The degree and extent of porosity depend on the tissue fluid concentration of fluoride during tooth development. Although the relationship of fluoride levels in drinking water to dental caries and dental fluorosis is known, the association between dental caries and fluorosis is not clear. This is simply due to the fact that only a few researchers have addressed this problem and the findings of such studies are conflicting. Some researchers have shown negative correlation between fluorosis and caries.[3, 4, 5] Some are of the opinion that fluorosed teeth are resistant to caries.[6] According to a number of authors no significant correlation was found between fluorosis score and DMFS Index.[7, 8] Some studies have shown positive correlation between caries and fluorosis.[9, 10] Therefore it would be appropriate to determine the correlation between fluorosis and caries so as to know about the effect of systemic fluorides. Hence the objectives of this study were:

1. Inducing the artificial caries in fluorotic and nonfluorotic teeth.
2. To compare the enamel thickness and depth of lesions in fluorotic teeth and nonfluorotic teeth.
3. To correlate the relationship between fluorosis and artificial caries.

MATERIALS AND METHOD

A total of 30 extracted permanent incisors were collected and divided into two groups, Group I: 15 extracted incisors effected by fluorosis (mild to moderate degree according to Dean's index) with no visual signs of caries or cracks were selected from areas with relatively high fluoride concentration in drinking water. Group II: 15 extracted incisors which were clinically normal with no visual signs of fluorosis,

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caries or cracks were collected from areas with normal fluoride concentration in drinking water. The extracted teeth were stored in 1% thymol solution until use. The teeth were cleaned to remove surface deposits. The crowns of the teeth were cut at the cementoenamel junction and the stump surface was covered with zinc phosphate cement. The crowns were coated with nail varnish all over except for windows measuring 2mmX 5mm on buccal surface. Thirty glass beakers were filled with 20ml of demineralizing solution (lactic acid with diphosphonate) at ph 5, and then each tooth was immersed in separate beakers which were individually labeled and placed in the incubator for 10 days at 37°c. After ten days the teeth were taken out and washed with double distilled deionised water. The teeth were ground buccolingualy using a hard tissue microtome by embedding them in cylindrical blocks of self cure acrylic resin. The teeth were mounted in such a way that either mesial or distal half was embedded in acrylic. The mounted tooth was placed in the hard tissue microtome and the tooth was sectioned parallel to the long axis of the tooth till ground sections of 100 microns were obtained. The mounted specimens were observed under polarized microscopy to measure the thickness of enamel and depth of the lesion. Each specimen was measured at three sites for lesion depth and enamel thickness and mean was taken. The mounted ground sections were viewed under Trinocular research microscope with polarizing microscope (Olympus BX51, Japan). The measurements were carried out on images captured using 3 chip CCD camera (Proview, Media Cybernetics, USA) with a 10x apochromatic objective. The resultant image on the monitor had a 500x final magnification and represented 0.168mm^2 of the tissue. All captured images were stored in hard disk and subsequently subjected for proper calibrations using the tools of Image–Proplus software V–4.1.0.0 (Media cybernetics, USA). For measuring the enamel thickness, the distance between outer surface of the enamel to dentinoenamel junction was considered and for depth of the lesion the distance between the outer surface of enamel to the deepest point of the lesion was considered. The software automatically calculated the thickness of enamel and depth of the lesion for all the specimens. All measurements were in microns. All the data obtained from image analysis software were exported to Microsoft excel master chart. Further interpretations and statistical analysis was done using student's unpaired t-test.

RESULTS

Comparison of thickness of enamel and depth of the lesions in fluorotic and nonfluorotic teeth was done. Both fluorotic and nonfluorotic group consists of 15 samples each. (Comparison of enamel thickness of fluorotic and nonfluorotic teeth of all 15 samples is shown in Table 1). Comparison of the mean score and standard deviation of enamel thickness of fluorotic and nonfluorotic teeth gives a t-value of -2.1496 and p-value of 0.0404 which is considered as statistically significant.

Table. 1 Comparison of fluorotic and non-fluorotic teeth groups with respect to Enamel Thickness

Group	n	Mean	SD	t-value	p-value	Sign.
Fluorotic teeth	15	572.1712	252.1926	-2.1496	0.0404	S
Non-fluorotic teeth	15	739.0524	163.7240			

Table 2. Comparison of fluorotic and non-fluorotic teeth groups with respect to depth of the lesion

Group	n	Mean	SD	t-value	p-value	Sign.
Fluorotic teeth	15	529.5127	133.5009	2.4298	0.0218	S
Non-fluorotic teeth	15	411.3569	132.8446			

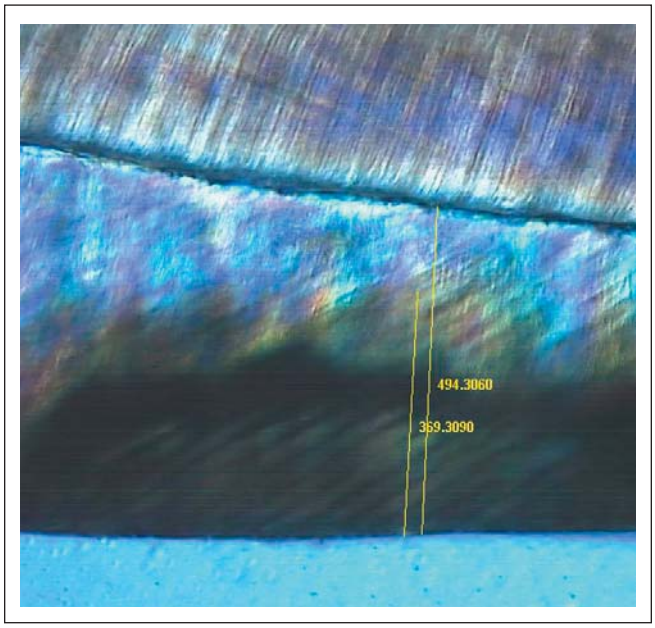

Figure 1. Polarized light microscopic view of fluorotic teeth group.

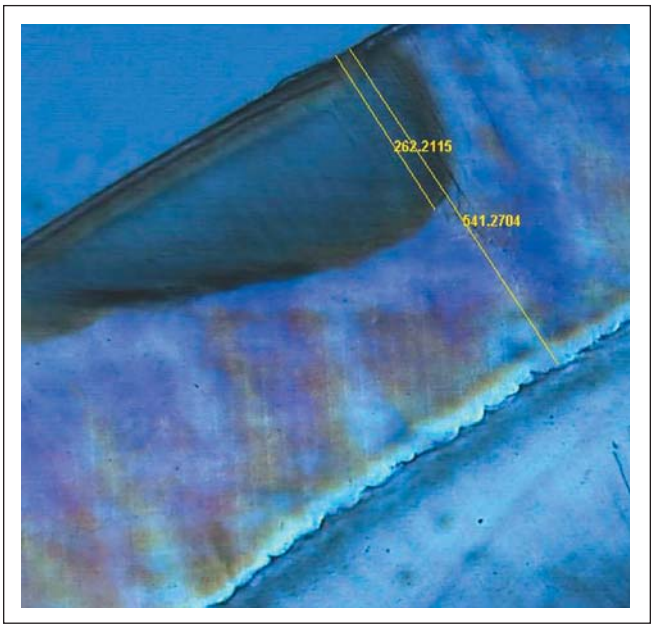

Figure 2. Polarized light microscopic view of nonfluorotic teeth group

(Comparison of depth of lesions of fluorotic and nonfluorotic teeth are shown in Table II) Comparison of the mean score and standard deviation of depth of lesion of fluorotic and nonfluorotic teeth gives a t-value of 2.4298 and p-value of 0.0218 which is considered as statistically significant. Statistical analysis showed that mean thickness of enamel of nonfluorotic teeth is significantly more when compared to fluorotic teeth and mean depth of lesion is significantly more in fluorosed teeth when compared to nonfluorosed teeth.

DISCUSSION

The current challenge is to develop new evidence based concepts in caries prevention through judicious use of fluorides. Though fluoride continues to be the cornerstone of caries prevention regime, if it exceeds the optimal level it leads to dental fluorosis which was commonly thought to result from the toxic effect of fluoride during the secretary phase of enamel formation leading to altered enamel structure and enamel surface hypoplasias.[12,13] A dogma had existed for many decades that fluoride has to be administered systemically and acts mainly during pre eruptive stage, however recent studies conclude that preventive effects of fluoride are almost exclusively topical. Though some studies show that caries prevalence is lower in groups of individuals with fluorosis than in nonfluorotic group, it is not because fluorosis prevents caries but because individuals with fluorosis will continue to benefit from the post eruptive effect of fluoride as long as they continue to live in an area with high water fluoride concentration.[14,15,16,17] Some authors have suggested that pre eruptive fluoride administration is ineffective and therefore unnecessary and indeed undesirable.[18] Today it is well known that the caries preventive effect of fluoride is almost cent percent posteruptive.[19] Although the relationship of fluoride level in drinking water to dental caries and dental fluorosis is known, the association between fluorosis and dental caries is still a matter of debate. So our study was aimed at evaluating the relationship between fluorosed teeth and caries and also to know about the thickness of enamel in fluorosed teeth.

Some of the previous studies done regarding the relationship between dental caries and dental fluorosis in endemic areas has shown that there is a systematic and positive relationship between caries and fluorosis.[8,9] Where has some authors found no significant correlation between fluorosis score and DMFS Index.[7] Contrary to these findings some studies have shown that mean DMFT value was less in high fluoride areas with maximum number of fluorosis cases when compared to low fluoride area with minimum or negligible number of fluorosis cases.[3] Studies done in Davangere region which is considered as one of the endemic fluoride area shows a low caries prevalence.[5] Some are of the opinion that maximum caries protection could be achieved with minimum enamel mottling.[20] This low prevalence of caries in endemic areas with maximum number of fluorosis cases may be due to the continued topical effect of fluorides. Results of this study showed that mean thickness of enamel in nonfluorotic teeth is significantly more when compared to fluorotic teeth and mean depth of lesion is significantly more in fluorosed teeth when compared to nonfluorosed teeth. This could be due to roughened enamel crystals[17] and defect in matrix formation in fluorosed teeth leading to reduced enamel thickness. The increased depth of lesion in fluorotic teeth is may be due to increased intercrystalline spaces and immature enamel crystals. Similar study conducted by Wondwossen et al revealed that there was a strong positive association between the Tooth Fluorosis and DMFT scores.[8] The DMFT scores differed significantly ($p< 0.05$) between children having Tooth Fluorosis scores 0 and those with Tooth Fluorosis scores 1-2, 3-4 and 5-7. Grobler also reported a positive correlation ($p < 0.05$) between caries experience and the fluorosis scores.[9]

Although fluoride is acknowledged as an essential component in the prevention of dental caries, systemic ingestion of fluoride has to be judiciously monitored on a regular basis to ensure that fluorosis does not occur. In a country like India due to high temperature, the consumption of drinking water is relatively high. Major source of drinking water in rural areas of India is ground water which is contaminated with excess fluoride in endemic area thereby leading to increased ingestion of fluoride. So community and domestic water defluoridation needs to be done in fluoride endemic areas.

CONCLUSION

The present findings in this *in vitro* study indicate that, dental caries increased and enamel thickness decreased with increased severity of dental fluorosis in fluoride endemic areas. Hence, the present results and those of a few previous studies strengthen the notion that there is a systematic and positive relationship between fluorosis and dental caries prevalence

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