The effect of fluorosis on human teeth under light microscopy: A cross-sectional study

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Abstract Background: Fluoride is needed for the normal development of bone and teeth; in high levels, it affects developing teeth and bone. Dental fluorosis (DF) is caused by ingestion of excess fluoride mainly through drinking water.

Aim: The present study aims to observe and understand the histological changes of fluorosed teeth under light microscope (LM).

Materials and Methods: Teeth which were indicated for extractions for orthodontic or periodontal problems were selected. Thirty extracted teeth were selected with varying degrees of DF based on modified Dean's fluorosis index. Ground sections of these teeth were prepared and the sections were studied under binocular LM. Photomicrographs were taken under high power objective using 15 megapixels Nikon camera.

Results and Conclusion: Qualitative histologic changes in different grades of fluorosed teeth were evaluated in enamel, dentin, cementum and between their junctions. Fluoride interacts with enamel in both mineral phases and organic macromolecules by strong ionic and hydrogen bonds resulting in incomplete crystal growth at prism peripheries. This presents as hypomineralization of enamel and dentin, increased interglobular dentin, increased secondary curvatures and changes in cementum such as diffuse cementodentinal junction and increased thickness of Tomes' granular layer. Changes in the structure of the teeth with Dean's index below 2 and teeth with Dean's index of 2 and above were compared using Chi-square test. *P* value was found to be highly significant being 0.00047. Many of the features of dental fluorosis seen in the present study under light microscope are comparable to those results studied under specialized microscopes.

Keywords: Dean's index, dental fluorosis, dentinoenamel junction, hole damage in enamel, hypomineralization, interglobular dentin, light microscopy, secondary curvatures

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INTRODUCTION

Dental fluorosis (DF) is caused by ingestion of excess fluoride mainly through drinking water, and Tamil Nadu, a

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southern state of India, has 10 out of 29 districts affected with fluorosis.^[1] Salem, a district in Tamil Nadu, is affected by both dental and skeletal fluorosis due to high levels of fluoride in drinking water. Although fluoride is needed for

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the normal development of bone and teeth, in high levels, it affects developing teeth and bone. This study elucidates and characterizes the changes of fluorosed human teeth collected from Salem by means of light microscope (LM).

MATERIALS AND METHODS

Ethical approval was obtained from Institutional Ethical Committee and written informed consent were obtained from the patients who took part in the study. Teeth which were indicated for extractions for orthodontic or periodontal problems were selected. Modified Dean's index was used as criteria for selection of teeth. They were obtained from people of age 15-40 years born and brought up in the district of Salem. Extracted fluorosed teeth were classified according to Dean s index. Teeth with caries and extrinsic stains were the exclusion criteria. Five teeth each with index scores 0, 0.5, 1, 2, 3 and 4 were selected. Ground sections were prepared by manual slicing of the teeth and grinding using carborundum stone. Finally, cleaned and dried sections were mounted on a clear glass slide and covered with a glass coverslip using dibutyl phthalate xylene. Photomicrographs of these ground sections were taken under LM-52-1702 binocular LM (N-120A) using 15 megapixels Nikon camera.

RESULTS

Coronal and radicular portions of the ground sections were viewed separately. Enamel with Dean's index 0 showed uniform striae of Retzius and uniform mineralization. Enamel in all the samples with fluorosis showed less mineralization. The data obtained were statistically analyzed using the SPSS software (version 11.5, IBM Corporation, Armonk, New York, USA); Chi-square test was used for statistical analysis.

CORONAL PORTION

Enamel

Figure 1 shows teeth with mild fluorosis; enamel showed thin areas which were white in the subsurface due to demineralization. In fluorosed teeth, the thickness of enamel was comparatively less and demineralization was seen as light-yellow and brown shade as shown. In Figure 2 fluorosis, mild demineralization was seen as light shade in the cuspal area of enamel with disturbance in gnarled enamel pattern. In moderate and severe fluorosis, the whiter areas in enamel were broader representing more areas of demineralization and they were evident in the deeper areas of the enamel as shown in Figures 3 and 4. In severe fluorosis, as seen in Figure 5, dentinoenamel junction (DEJ) was not scalloped but irregular. Intermingling of crystals



Figure 1: Enamel showed thin areas which were white in the subsurface due to demineralization



Figure 2: Mild demineralisation in DEJ in cusp and disturbance in gnarled enamel pattern

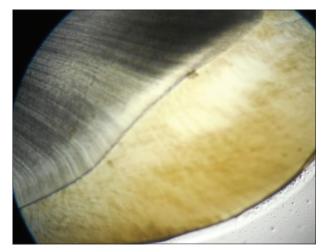


Figure 3: Moderate Dental fluorosis - whiter areas becoming broader representing more areas of demineralization in deeper areas

was seen in the junction between dentin and enamel. In sections of moderate and severe fluorosis, broadening of

incremental lines was seen [Figure 6]. Figure 7 shows severe demineralization, and hole damage was seen in enamel with Grade 4 DF.

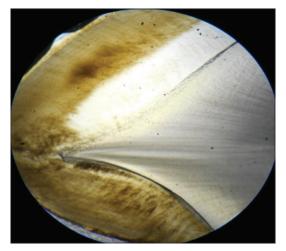


Figure 4: Moderate demineralization near dentinoenamel junction

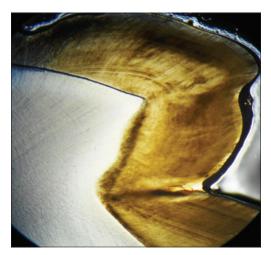


Figure 6: Moderate and Severe with darker and broader incremental lines

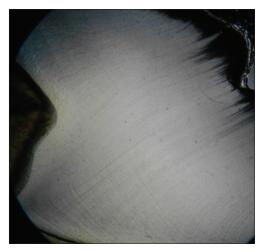


Figure 8: Normal dentinal tubules

Dentin

In Figure 8, dentinal tubules without fluorosis showed a gentle curve in the crown resembling "S" which is less in the root called as primary curvatures. The dentinal tubules



Figure 5: Severe dental fluorosis

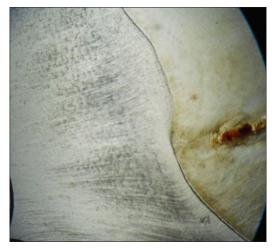


Figure 7: Severe dental fluorosis with hole damage seen in enamel

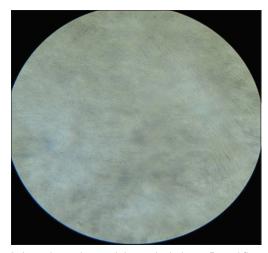


Figure 9: Irregular and curved dentinal tubules in Dental fluorosis

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were more irregular and curved in fluorosed teeth as shown in Figure 9. Some of the fluorosed teeth with higher grades showed more amount of interglobular dentin as shown in Figure 10 with numerous dead tracts as shown in Figure 11. Figure 12 shows secondary curvatures which were found to be numerous in fluorosed teeth.

Radicular portion

The cementodential junction was diffuse and irregular in higher grades of fluorosis as shown in Figure 13. Figure 14 shows fluorosed teeth with higher grades with increased thickness of Tomes' granular layer.

Out of the 30 teeth studied by light microscopy, 16 teeth showed changes in enamel, 11 showed changes in dentin, 7 showed changes in cementum, 10 showed changes in DEJ and 5 showed changes in cementoenamel junction [Table 1 and Graph 1]. Changes in the structure of the teeth with Dean's index below 2 and teeth with Dean's index of 2 and above were compared using Chi-square test. *P* value was found to be highly significant being 0.00047 [Table 2].



Figure 10: More interglobular dentin

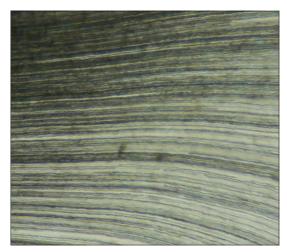


Figure 12: Dental fluorosis with numerous secondary curvatures

DISCUSSION

DF is characterized by opaque, lusterless white patches in the enamel which may become striated, pitted and discolored with a breakdown of mineralized layer shortly after eruption.^[2] Opacity results from incomplete crystal growth in enamel in teeth with DE^[3]

With increasing severity of fluorosis, the subsurface enamel all along the tooth becomes increasingly porous (hypomineralized), and the lesion extends toward the inner enamel.^[4] In our study, this finding was evident.

It has been reported that there is extensive abrasion of the porous and soft exposed lesions in severe fluorotic enamel because when the well-mineralized surface zone is fractured away, the exposed hypomineralized lesions caused by extensive modifications in oral environment.^[4] Histological examination of teeth from areas of high fluoride concentration showed more alterations in the

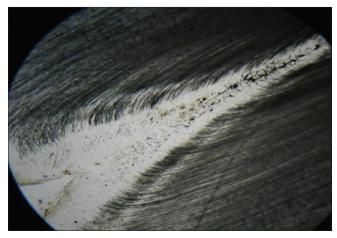


Figure 11: Dental fluorosis with more dead tracts

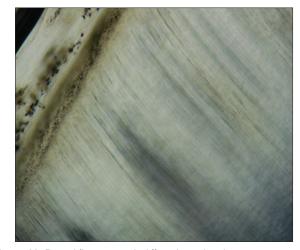


Figure 13: Dental fluorosis with diffuse boundary between cementum and dentin

enamel than that of dentin.^[5] This was present in our present study also.

This suggests that fluoride affects the processes involved in biomineralization, in general, no matter if the crystal formation and growth occur either in mesenchymal or ectodermally derived mineralized tissues,^[3] when higher concentrations of fluoride like 5 ppm can cause mottling and hypoplasia of the enamel and hypomineralized dentin and increased interglobular spaces.^[6]

It has been reported that the enamel surface of fluorosed teeth appeared highly uneven and rough showing cracks and fissures. The enamel surface showed pits of varying dimensions in the discolored area of the teeth and these appear as punched lesions on the enamel surface, thus exposing the underlying porous enamel.^[7] Considering the complexity of the biological mineralization process, the exact mechanism leading to DF is not fully understood.^[8]



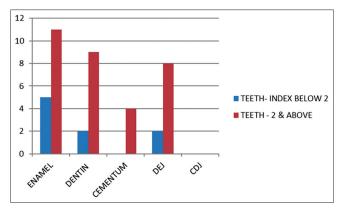
Figure 14: F Teeth - Wider Tom's Granular layer

In an optical and scanning electron microscopic study of fluorosed teeth, the borders between enamel and dentin and the border between dentin and cementum were obscure and uneven, and part of the enamel was damaged.^[9] This was evident in severe grades of DF in our study also.

In the longitudinal section of fluorosed incisor tooth from goats in an industrially polluted area, there was an obvious hole damage extending from the outer layer of the enamel into the dentine area.^[10] It was present in higher grades of DF in our study also.

A recent study on ground sections of fluorosed teeth reported crescent-shaped hypomineralized areas in enamel and increased interglobular dentin spaces.^[11] Similar features were seen in our study also. Many of the features of dental fluorosis seen in the present study under light microscope are comparable to those results studied under specialized microscopes.

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Graph 1: Comparison of light microscopic changes in teeth structures

Serial number	Dean's index	Number of teeth	Enamel	Dentin	Cementum	Dentinoenamel junction	Cementodentinal junction
1	0	5	2	0	0	0	0
2	0.5	5	1	1	0	1	0
3	1	5	2	1	0	1	0
4	2	5	2	1	0	1	1
5	3	5	4	4	4	3	2
6	4	5	5	4	3	4	2
Total		30	16	11	7	10	5

Table 1: Comparison of Dean's index and changes in the structure of the teeth

Table 2: Statistical analysis using Chi-square test

	Enamel	Dentin	Cementum	Dentinoenamel junction	Cementodentinal junction	Chi-square test
Dean's index below 2	5	2	0	2	0	P=0.00047609
Dean's index - 2 and above	11	9	4	8	5	

P value is highly significant being 0.00047

CONCLUSION

Fluoride interacts with both mineral phases and organic macromolecules by strong ionic and hydrogen bonds resulting in incomplete crystal growth at prism peripheries. This presents as hypomineralization of enamel and dentin and change in the microscopic structure of teeth.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Ramesh M, Narasimhan M, Krishnan R, Chalakkal P, Aruna RM, Kuruvilah S, *et al.* The prevalence of dental fluorosis and its associated factors in Salem district. Contemp Clin Dent 2016;7:203-8.
- Fejerskov O, Johnson NW, Silverstone LM. The ultrastructure of fluorosed human dental enamel. Scand J Dent Res 1974;82:357-72.

- Robinson C, Connell S, Kirkham J, Brookes SJ, Shore RC, Smith AM, et al. The effect of fluoride on the developing tooth. Caries Res 2004;38:268-76.
- Fejerskov O, Manji F, Baelum V. The nature and mechanisms of dental fluorosis in man. J Dent Res 1990;69:692-700.
- Burt BA, Fejerskov O. Water fluoridation. In: Fejeskov O, Ekstrand J, Burt BA, editors. Fluoride in Dentistry. Copenhagen: Munksgaard; 1996. p. 275-90.
- Richards A, Kragstrup J, Josephsen K, Fejerskov O. Dental fluorosis developed in post-secretory enamel. J Dent Res 1986;65:1406-9.
- Susheela AK, Bhatnagar M. Structural aberrations in fluorosed human teeth: Biochemical and scanning electron microscopic studies. Current Sci 1999;77:1-6.
- Thylstrup A, Fejerskov O. Clinical appearance of dental fluorosis in permanent teeth in relation to histologic changes. Community Dent Oral Epidemiol 1978;6:315-28.
- Wang J, Hong J, Li J, Guo Y, Zhang J, Hao J. Effect of high fluoride and low protein on tooth matrix development in goats. Fluoride 2002;35:51-5.
- Wang J, Guo Y, Liang Z, Hao J. Amino acid composition and histopathology of goat teeth in an industrial fluoride polluted area. Fluoride 2003;36:177-84.
- Priyadharsini N, Malathi N, Tamizhchelvan H, Dineshkumar T. Dental fluorosis: A histological study using light and confocal microscopy. Indian J Dent Res 2015;26:248-51.