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International Journal of Current Research
Vol. 8, Issue, 11, pp.42460-42465, November, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

COMPARATIVE EVALUATION OF DENTINAL SURFACE CHANGES USING TETRACYCLINE HYDROCHLORIDE AND ETHYLENEDIAMINETETRAACETIC ACID IN FLUOROSED AND NON-FLUOROSED TEETH WITH SCANNING ELECTRON MICROSCOPE-AN INVITRO STUDY

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ARTICLE INFO

Article History:
Received 07th August, 2016
Received in revised form
20th September, 2016
Accepted 24th October, 2016
Published online 30th November, 2016

Key words:

Fluorosis, Root biomodification agents,
Degree of demineralization,
EDTA, Tetracycline hydrochloride.

ABSTRACT

Background: Fluorosis may change the mineralisation of the teeth. For this the first study was done by Vandana K L in 2007 to assess the dentinal surface changes in fluorosed teeth after chemical root biomodification.
Aim: To compare dentinal surface changes in fluorosed and non-fluorosed teeth after application of EDTA and Tetracycline Hydrochloride.
Materials and Methods: Thirty two human teeth extracted due to advanced periodontal disease and orthodontic treatment planning was used in this study. Also thirty two fluorosed teeth extracted due to periodontal disease and orthodontic treatment planning was used. The teeth were scaled and root planed. Sixty four dentin discs were prepared and divided into four groups like fluorosed healthy, non-fluorosed healthy, fluorosed diseased and non-fluorosed diseased. After, they were treated with EDTA and Tetracycline Hydrochloride. All the specimens were fixed and observed under scanning electron microscope and statistically analysed.
Results: Overall smear layer score was more in FH group followed by NFH, FD, and NFD group in descending order with the specimen treated with TTC HCL. For degree of demineralization it was higher in FH followed by NFH and NFD were same and FD with specimen treated with TTC HCL.
Conclusion: Fluorosed teeth show more degree of demineralization than non-fluorosed teeth.

Citation: Dr. Malathi, K., Dr. Dhanesh Sabale, Dr. Muthukumarswamy, A. Dr. Shabbir, A. and Dr. Dnyaneshwar Punse. 2016. "*Comparative evaluation of dentinal surface changes using tetracycline hydrochloride and ethylenediaminetetraacetic acid in fluorosed and non-fluorosed teeth with scanning electron microscope- An invitro study*", *International Journal of Current Research*, 8, (11), 42460-42465.

INTRODUCTION

As the fluoride has affinity to Ca+++, excess of fluoride causes dental fluorosis if the individual gets affected during tooth developmental period. In periodontitis the affected tooth surface is hypermineralised due to action of cytotoxic and biologically active contaminants. On such surfaces proliferation of adjacent cells for the purpose of periodontal healing is hampered due to diseased materials on it (Delazari, 1999; Blomlof *et al*., 1995). Mechanical instrumentation can remove this diseased material from the root surface but can form the smear layer of organic and inorganic debris. For this purpose various chemical root biomodification materials has been used. Tetracycline, EDTA has added effect of removing the smear layer to expose collagen matrix of the mineralized radicular tissue, which enhances the fibroblastic attachment (Terranova, 1986).

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The fluorosed population is bound to experience more periodontal disease. Dental fluorosis causes hypomineralizaton of enamel and dentin. The effect of fluorosis on the cementum is not clear. The routine periodontal treatment in fluorosed patients has raised the question, that whether the root conditioning effects in fluorosed teeth would remain same or different in comparing with non-fluorosed teeth. Few reports show that there is no clinically significant benefit of treating root surfaces with chemical conditioners. The effects of root conditioning agents are not consistent in periodontal literature. But, root biomodification as a treatment modality is not outdating yet. From routine clinical observation association between periodontitis and high fluoride content water areas were observed (Vandana and Reddy, 2007). SEM observations shows there is high globular mineralised debris and mineralization of periodontal ligament areas in fluorosed healthy teeth group as compared to non- fluorosed group (Hanes *et al*., 1989). Hence, in this study using Scanning electron microscope for the evaluation of smear layer and collagen exposure in dentin specimens of fluorosed and non-fluorosed teeth which comprised of periodontally healthy and

diseased teeth, subsequent to the application of Tetracycline HCL (TTC) and EDTA is experimented.

MATERIALS AND METHODS

This in-vitro study consists of sixty four extracted periodontally compromised and healthy, fluorosed and non-fluorosed teeth, suitable to the selection criteria, obtained from the Department of Oral Maxillofacial Surgery, Tamil Nadu Government Dental College and Hospital.

Inclusion Criteria: (1) Were to be fully erupted, (2) Extracted non-traumatically due to orthodontic reasons, (3) No history of recent periodontal instrumentation or dental prophylaxis, (4) Periodontally diseased teeth with at least 60% attachment loss indicated for extraction.

The Exclusion Criteria: (1) Teeth with proximal caries extending to the cementum, (2) Fillings extending beyond CEJ, and (3) Intrinsic stains caused by other reasons such as porphyria, erythroblastosis fetalis, tetracycline therapy etc.

Scanning Electron Microscopic Examination

- 4% Glutaraldehyde (pH 7.2)
- Phosphate buffered saline (PBS) pH 7.2
- Aqueous ethanol (25, 50, 75, 100%)
- Gold sputtering unit (Hitachi E-1010 Ion Sputter)
- Scanning Electron Microscope (Hitachi S-3400 N)

Procedural Steps: Dentin disc specimen preparation.

Periodontally Healthy Fluorosed and Non-Fluorosed Teeth

- The root surfaces were hand instrumented, using a sharp periodontal curette to remove the remnants of periodontal ligament without resulting in total removal of cementum, by using 12 strokes approximately.
- Coronal section was then prepared 1 mm below the cemento- enamel junction and apical section 3mm from the root apex, using a sterile diamond disk running at low speed with sterile water coolant.
- Then a longitudinal section was performed to obtain 2specimens from each root representing dentin and cementum specimen. The two halves were instrumented; using both hand and rotary (fine diamond tapered bur) instruments to expose the dentine and were washed in water.
- Dentin specimens were equally distributed into TTC HCL, EDTA groups for root conditioning procedures.

Periodontally Diseased Fluorosed and Non-Fluorosed Teeth

- A reference groove was marked on the root surface at the level of soft tissue attachment. The root surface was instrumented, using a sharp periodontal curette to remove the remnants of periodontal ligament, calculus and superficial cemental layer coronal to the level of marking, using 50 strokes approximately.
- The anatomical crown, including 1 mm of the coronal portion of the root, was resected with a high speed diamond disc.
- Dentin specimens were taken from the middle one-third of the root of periodontitis-affected human root surfaces.
- The root portion coronal to the level of groove was retained and the portion apical to the groove was discarded.
- The preparations of dentin specimens for periodontally diseased teeth roots were done in the same way as for the periodontally healthy root specimens.
- Dentin specimens were equally distributed into TTC HCL, EDTA groups for root conditioning procedures.

Experimental Groups and Treatments

Sixty four dentin specimens were taken and divided into three groups.

Group I (FH): 16 specimens were treated with TTC HCL and 16 were treated with EDTA solution for biomodification.
Group II (FD): 16 specimens were treated with TTC HCL and 16 were treated with EDTA solution for biomodification.
Group III (NFH): 16 specimens were treated with TTC HCL and 16 were treated with EDTA solution for biomodification.
Group IV (NFD): 16 specimens were treated with TTC HCL and 16 were treated with EDTA solution for biomodification.
Then the specimens were kept in separate petri dishes with denoting their names respectively.

Root Surface Treatment: In this study, a concentration of 500mg/5ml (100mg/ml), pH 1.8 of Tetracycline HCL, 24% EDTA pH 7.4, were used. The specimens were burnished with solution-saturated cotton pellet with respective agents in each group for 3 minutes. Pellets were changed at every 30-second intervals, and specimens were then rinsed under running tap water.

Preparation for scanning electron microscopic examination (SEM)

- The specimens were fixed in freshly prepared 4% glutaraldehyde solution (pH 7.2) at room temperature for 24 hours and washed thrice with DPBS for 10 minutes .
- The specimens were then dehydrated in graded series of aqueous ethanol (25, 50, 75, and 100%) for 10 minutes at each concentration.
- The specimens were then dried in a desiccator for 48 hours.
- The specimens were then sputter coated with 200 A° of gold using a sputter coater (Hitachi E-1010 Ion Sputter).
- The specimens were mounted in scanning electron microscope stubs and examined in Scanning Electron Microscope (Hitachi S-3400 N) operating at an accelerated voltage of 15-20 kV.
- All specimens were viewed at a standard magnification of 1500x and 3500x.
- Then the score for smear layer and degree of demineralization were calculated. The data collected was statistically analysed.

Study Parameters

- Score of smear layer according to scoring criteria.

- Score of degree of demineralization according to scoring criteria.

The dentinal SEM photographs were scored for smear layer using following scoring criteria (Vandana *et al.*)

0 = None
1 = Smear layer involving random areas of surface that totals between 1-32% of total surface area.
2 = Smear layer involving random areas of surface that totals between 33-65% of total surface area.
3 = Smear layer involving > 66% of total surface area. Assessment of degree of

Demineralization of Dentinal specimens was scored using following criteria (Vandana *et al.*)

0= None
1= (Slight) Localized exposure of matrix collagen with an evidence of acid denaturing and possible widening of a few dentinal tubule orifices.
2= (Moderate) Localized or generalized exposure of matrix collagen with an evidence of acid denaturing, obvious widening of dentinal tubule orifices, and evidence of peritubular dentin demineralization.
3= (Severe) Generalized exposure of matrix collagen with severe acid denaturing, gross widening of dentinal tubule orifices, and obvious peritubular dentin demineralization.

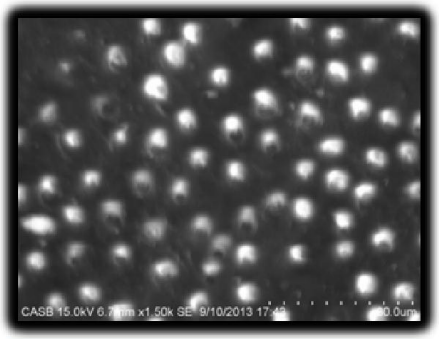
RESULTS

Comparison of smear layer score in ttc hcl and edta Group

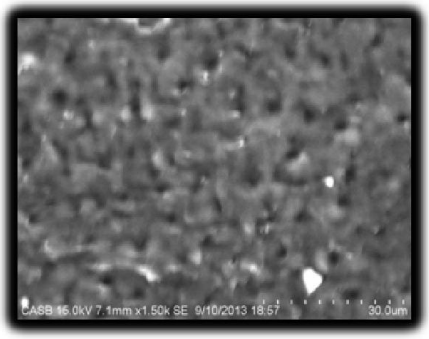
On comparison of the mean of smear layer scores, for FH sample in TTC HCL group the mean was 1.5± 1.096 higher than that of EDTA group (0.88± 0.991) however the value was not statistically significant (p=0. 245). In FD group comparison of mean of smear layer score was 1.00± 0.926 for TTC HCL and for EDTA group it was 0.50± 0.756, which shows that TTC HCL group had higher score of smear layer (p=0.256) however the value stood not statistically significant. On comparison of the mean of smear layer scores, for NFH group it was 1.25± 0.707 for TTC group and for EDTA group it was 0.88± 0.64 indicates again TTC was greater than EDTA (p=0.285) all the same the value be situated not statistically significant.But in NFD group the mean of smear layer score was 0.13± 0.354 with TTC HCL treatment, where in EDTA group it was 1.13± 0.354. In this group EDTA was having significantly higher smear level score than TTC HCL. (p= 0.000).

Comparison of degree of demineralization

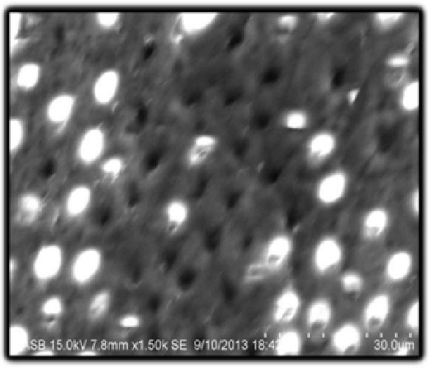
In the present study, TTC HCL group degree of demineralization in FH sample were mean of 1.38± 0.744, which was higher than EDTA group 0.50± 0.926. (p= 0.056) although the value was not statistically significant.



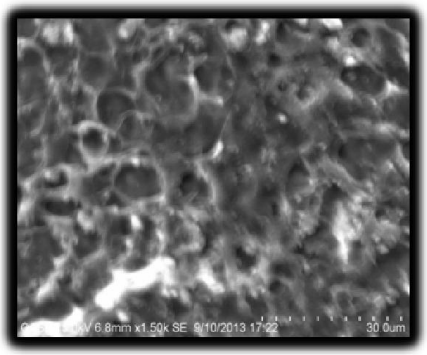
PHOTOMICROGRAPH 1: FLUOROSED DISEASED TETRACYCLINE HYDROCHLORIDE TREATED TOOTH SAMPLE



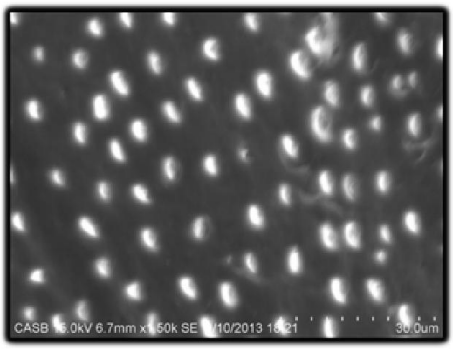
PHOTOMICROGRAPH 2: FLUOROSED DISEASED EDTA TREATED TOOTH SAMPLE



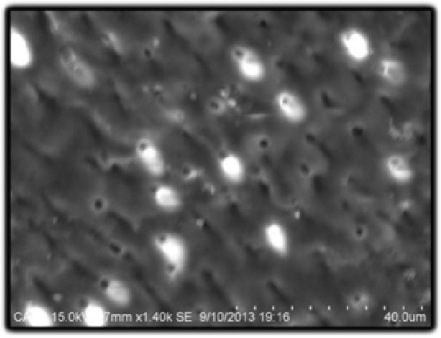
PHOTOMICROGRAPH 3: FLUOROSED HEALTHY EDTA TREATED TOOTH SAMPLE



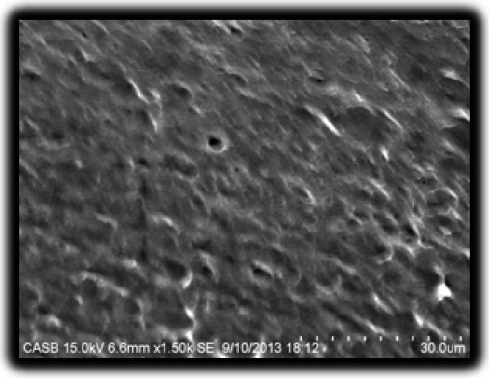
PHOTOMICROGRAPH 4: FLUOROSED HEALTHY TETRACYCLINE TREATED TOOTH SAMPLE



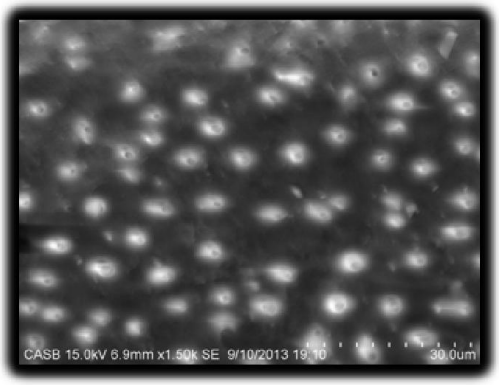
PHOTOMICROGRAPH 5: NON-FLUOROSED DISEASED TETRACYCLINE HYDROCHLORIDE TREATED TOOTH SAMPLE



PHOTOMICROGRAPH 6: NON-FLUOROSED DISEASED EDTA TREATED TOOTH SAMPLE



PHOTOMICROGRAPH 7: NON-FLUOROSED HEALTHY TETRACYCLINE TREATED TOOTH SAMPLE



PHOTOMICROGRAPH 8: NON-FLUOROSED HEALTHY EDTA TREATED TOOTH SAMPLE

In FD group the degree of demineralization was 0.88± 0.835 for TTC HCL which was higher than EDTA group which was having 0.38± 0.518. (p=0.172). For NFH group degree of demineralization of TTC HCL group was (1.25± 0.463) statistically significantly higher than EDTA group 0.38± 0.518. (p=0.003). In NFD group degree of demineralization with TTC HCL treatment was 1.25± 0.707, wherein EDTA group, it was 0.25± 0.463 (p=0.005) with the significant value.

Comparison of percentage of each smear layer score in each sample when treated with ttc & EDTA

Pearson's Chi square test was used for the comparison of percentage of each smear layer score when treated with TTC HCL & EDTA. In FH sample the comparison of % revealed 25% of the tooth when treated with TTC HCL reported highest smear layer score (i.e. score 3) whereas only 12.5% of the tooth reported highest smear layer, when treated with EDTA though the p value was not statistically significant (p= 0.506).
In FD sample the comparison of % discovered 37.5% of the tooth when treated with TTC HCL reported highest smear layer score (i.e. score 2) while only 12.5% of the tooth reported chief smear layer, when treated with EDTA though the p value was statistically insignificant (p= 0. 472). In NFH sample the comparison of % shown 37.5% of the tooth when treated with TTC HCL described uppermost smear layer score (i.e. score 2) whereas only 12.5% of the tooth reported highest smear layer, when treated with EDTA though the p value was not statistically significant (p= 0.486). In NFD sample the comparison of % revealed 0% of the tooth when treated with TTC HCL conveyed highest smear layer score (i.e. score 2) whereas only 12.5% of the tooth testified highest smear layer, when treated with EDTA though the p value was statistically significant (p= 0.002).

Comparison of percentage of each degree of demineralization in each sample when treated with ttc & EDTA

In FH sample the comparison of % of highest degree of demineralization score revealed 50% of the tooth when treated with TTC HCL reported highest degree of demineralization (i.e. score 2) whereas only 25% of the tooth reported highest degree of mineralization, when treated with EDTA (p= 0.027). The p value was statistically significant. In FD sample the comparison of % revealed 25% of the tooth when treated with TTC HCL reported maximum degree of demineralization (i.e. score 2) whereas only 0% of the tooth reported highest degree of mineralization, when treated with EDTA (p= 0.287). The p value was not statistically significant. In NFH sample the comparison of % revealed 25% of the tooth when treated with TTC HCL reported highest degree of demineralization (i.e.

score 2) however only 0% of the tooth reported maximum degree of mineralization, when treated with EDTA (p= 0.018). The p value was statistically significant. In NFD sample the comparison of % revealed 37.5% of the tooth when treated with TTC HCL reported highest degree of demineralization (i.e. score 2) while only 0% of the tooth reported maximum degree of mineralization, when treated with EDTA (p= 0.027). The p value was statistically significant.

Tables 1. Smear layer score Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
FH	TTC	8	1.50	1.069	.378
	EDTA	8	.88	.991	.350
FD	TTC	8	1.00	.926	.327
	EDTA	8	.50	.756	.267
NFH	TTC	8	1.25	.707	.250
	EDTA	8	.88	.641	.227
	TTC	8	.13	.354	.125
NFD	EDTA	8	1.13	.354	.125

Table 2. Evidence of demineralization group statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
FH	TTC	8	1.38	.744	.263
	EDTA	8	.50	.926	.327
FD	TTC	8	.88	.835	.295
	EDTA	8	.38	.518	.183
NFH	TTC	8	1.25	.463	.164
	EDTA	8	.38	.518	.183
NFD	TTC	8	1.25	.707	.250
	EDTA	8	.25	.463	.164

DISCUSSION

In this study, both fluorosed and non-fluorosed, periodontallyhealthy and diseased teeth were included. In periodontallyhealthy teeth, 12 strokes were given to remove theremnants of periodontal ligament fibers by using sharp curette (Hanes, 1980), and in diseased teeth approximately 50 strokesweregiven to remove remnants of periodontal ligament fibres, calculus and superficial cemental layer (Trombelli, 1995). Root planning was done in order to enhance the action of root conditioningagents. For the dentinspecimens, the tooth was vigorouslyroot planed with hand curettes and finishing burs inhigh speed hand pieces, in an attempt to remove all the cementumand to achieve a smooth hard glass-like surface ofdentin (Lafferty, 1993). In this study, periodontally affected root surface has high content of calcium, phosphorous and fluoride, and hypermineralization has been seen in superficial 40µm-100 µm area of root surface (Trombelli, 1995). This diseased root surface causes the enzymatic activity in periodontal pocket (Trombelli, 1996). In this study, the extracted teeth were stored in 0.9% normal saline. However, other storage media such as 5% phosphate buffered saline solution, 10% formalin, Hank's balanced salt solution and 0.1% sodium cacaodylate buffer had also been used in various studies (Bouchard, 1997).

Comparison between fluorosed teeth and non-fluorosed teeth with the treatment of ttc hcl and edta

In the present study, FH group smear layer score with treatment of TTC HCL showed the mean of 1.5, wherein EDTA group it was 0.88. In NFH group smear layer score with TTC HCL treatment was mean of 1.25 and with EDTA group it was 0.87, which resembled the results of previous study by Vandana *et al*. This overall interpretation shows that fluorosed healthy teeth have more tendencies for demineralization by treatment with TTC HCL. In FD group, smear layer score with TTC HCL was 1.0, wherein EDTA group it was 0.5, and in NFD group smear layer score was 0.13 with treatment of TTC HCL which was less than EDTA group score of 1.13. This interpretation showed that only among EDTA group NFD showed more propensities for demineralization than TTC HCL NFD group.

Comparison within the ttc hcl and edta group

Overall smear layer score was more in FH group followed by NFH, FD, and NFD group in descending order with the specimen treated with TTC HCL. This resembles the previous study done by Vandana *et al*. For degree of demineralization it was higher in FH followed by NFH and NFD were same and FD with specimen treated with TTC HCL. In this study smear layer score was more in NFD group of EDTA treated teeth samples, which was opposite to the interpretation in TTC HCL group where NFD showed least number of smear layer score. So, for EDTA group the sequence was NFD >FH> NFH>FD, which again revels that NFD teeth shows more degree of demineralization among the teeth treated with EDTA as a root biomodification agent.

Comparison of percentage of each smear layer score in fluorosed and non-fluorosed teeth sample (both healthy and diseased) when treated with ttc & edta

In FH sample the comparison percentage of highest smear layer score revealed that TTC HCL has more percentage of smear layers with highest score of 3 (25%) than EDTA (12.5%). In NFH sample, the comparison percentage of highest smear layer score indicates 37.5% of the smear layer when treated with TTC HCL described uppermost smear layer score (i.e. score 2), whereas only 12.5% of smear layer reported highest smear layer score, when treated with EDTA. That resembles to previous study did by Vandana *et al*. On comparing FD group with NFD group with the treatment of TTC HCL and EDTA the percentage of smear layer score in each smear layer score shows highest within FD group. So, it implies that fluorosed teeth show more percentage of highest smear layer score than the non-fluorosed teeth.

Comparison of percentage of each degree of demineralization score in fluorosed and non-fluorosed (both healthy and diseased) sample when treated with ttc & edta

In FH sample percentage of highest degree of demineralization score, which was 50% in TTC HCL and 25% in EDTA group for score 2 was more than the percentage of highest degree of demineralization in NFH group, which was 25% in TTC HCL group and 0% in EDTA group. The percentage of highest degree of demineralization in FD group, which was 25% in TTC HCL group and 0% in EDTA group was more than percentage of highest degree of demineralization in NFD group which was 37.5% for TTC HCL and 0% for EDTA for score 2.To interpret this, clearly the fluorosed teeth whether it is healthy or diseased had more demineralization capacity than non-fluorosed healthy or diseased teeth.

Summary and Conclusion

In the present study, 16 fluorosed teeth extracted due to advanced periodontal disease, 16 fluorosed teeth extracted due

to orthodontic treatment, 16 teeth extracted due to advanced periodontal disease and 16 teeth extracted due to orthodontic treatment were included. These teeth were cleaned, scaling and root planing was performed. 64 dentin disc of 2mm thickness were prepared and divided into the 4 groups; Group1 – Fluorosed healthy (FH), Fluorosed diseased (FD), Non-fluorosed healthy (NFH) and Non-fluorosed diseased (NFD). All four groups again equally divided into Tetracycline hydrochloride group and EDTA group. After the treatment the test specimens were fixed for SEM analysis. The specimens were viewed at 1500x to 3000x. The SEM microphotographs were assessed for score of smear layer and degree of demineralization.

The following result was obtained from the data acquired and the statistical analysis performed:

- Fluorosed teeth whether it is diseased or healthy showed more tendency for demineralization than non-fluorosed diseased or healthy teeth.
- Within FH, FD, NFH group TTC HCL showed more degree of demineralization than EDTA. But in NFD group EDTA showed more degree of demineralization than TTC HCL.
- Within TTC HCL treated teeth specimen sample, degree of demineralization was more in FH group followed by NFH, FD, and NFD. And, within EDTA group NFD group showed more degree of demineralization followed by FH, NFH and FD groups.

Within the limitations of the present in vitro study, it can be concluded that TTC HCL can be more effective in demineralization of fluorosed teeth after root biomodification but not much noteworthy than EDTA. Further studies are needed, with more number of teeth samples and different concentration of root biomodification agents.

Abbreviations

TTC = Tetracycline hydrochloride
EDTA = Ethylenediaminetetraacetic acid
CA = Citric acid
SEM = Scanning electron microscope
FH = Fluorosed healthy
NFH = Non-fluorosed healthy
FD = Fluorosed diseased
NFD = Non-fluorosed diseased
CEJ = Cemento-enamel junction

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