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## **Mineral content of nonfluorosed and fluorosed dental cementum – An *in vitro* study**

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### **Abstract**

**Background & Objectives:** The literature on effect of fluoride on dental caries is well discussed in contrast to periodontal tissues. However a recent review has explored an epidemiological association between fluorosis and periodontal disease and also the influence of periodontal treatment on fluorosed and non fluorosed teeth. There is a scarcity in literature dealing with effect of fluorosis on biological tissues like cementum. During progression of periodontitis, there is a possibility of micro hardness, mineral and histological changes in cementum. Considering the higher incidence of periodontitis in endemic fluorosed area around Davangere, there is an opportunity to study the cemental changes due to fluorosis which would influence the initiation and progression of periodontal disease. Hence the aim was to study the mineral content of fluorosed and nonfluorosed cementum.

**Materials and Methods:** A total of 24 healthy nonfluorosed and fluorosed orthodontically extracted premolars were collected to assess and compare the mineral content of fluorosed versus non fluorosed cementum using energy dispersive spectroscopy (EDS).

**Results and Conclusion:** The results of the study showed that the percentage of calcium ( $60.73 \pm 2.15$ ), magnesium ( $0.47 \pm 0.17$ ) and phosphorous ( $23.95 \pm 0.47$ ) was higher in nonfluorosed group than in fluorosed group. The percentage of fluoride ( $0.11 \pm 0.10$ ), sodium ( $0.37 \pm 0.09$ ), zinc ( $2.94 \pm 0.91$ ), potassium ( $0.40 \pm 0.15$ ), carbonate ( $10.74 \pm 2.56$ ) was higher in fluorosed group than in nonfluorosed group.

**Keywords:** Dental fluorosis, periodontitis, dental caries, mineral content, dental cementum.

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### **1. Introduction**

Fluorine is a common element in the earth's crust and is an essential element for the calcification of bones and teeth. Fluoride ion has played a major role in dramatically reducing dental caries over past 40 years. Excessive systemic exposure to fluoride can lead to disturbances of bone homeostasis, enamel development [dental/ enamel fluorosis] and mineralization. The severity of fluorosis on periodontal hard and soft tissues is dose dependent and also depends on timing and duration of fluoride exposure during development.[1]

The literature on effect of fluoride on dental caries is well discussed in contrast to periodontal tissues. However, fifteen years of research and a recent review by Vandana K L has presented an epidemiological association between fluorosis and periodontal disease, but also the influence of fluorosis on periodontal structures along with the comparison of influence of periodontal treatment on fluorosed and non fluorosed teeth. There is a scarcity in

literature dealing with effect of fluorosis on biological tissues like bone and cementum. [2]

During progression of periodontitis, there is a possibility of mechanical, physical and chemical changes in cementum. Considering the higher incidence of periodontitis in endemic fluorosed area around Davangere, there is an opportunity to study the cemental changes due to fluorosis which would influence the initiation and progression of periodontal disease.

Mineral content of fluorosed cementum have been studied scantily. Fluoride concentrations seem in general to be higher in cementum than in other mineralized tissues. There could be several reasons for this. Cementum is more permeable than other mineralized tissues and so will probably be more accessible to fluoride present in the fluid around periodontal ligament. Its high metabolic activity will facilitate fluoride uptake which will also depend on the rate of cementum deposition. This and

fluctuations in the amounts of fluoride ingested, will contribute to the characteristic fluoride profiles [3].

The question of whether any changes in physical properties or chemical composition of cementum take place prior to, or as a result of, periodontal disease has been a subject of disagreement in the dental literature. It has been the opinion of many clinicians that alterations occur in cementum and even dentin, during periodontal disease, resulting in an infected or softened root surface [4]. The available information leaves some uncertainty as to whether alterations associated with periodontal disease occur in cementum and root dentin. It seems likely, however, that if any changes take place, they involve the mineral content of these tissues.

The mineral content which may be different in fluorosed cementum would influence the pathogenesis of periodontal disease and /or outcome of periodontal treatment. Hence, the comparison of fluorosed versus nonfluorosed cementum is a new area of interest in fluorosis research. Medline search using keywords fluorosed and nonfluorosed cementum does not reveal much data. So present study aims to find out changes in mineral content of fluorosed versus non fluorosed cementum.

## 2. Materials and Methods

A total of 24 healthy nonfluorosed and fluorosed orthodontically extracted premolars were obtained from Department of Oral and Maxillofacial Surgery, College of dental sciences, Davangere. Subjects with age group of 18 to 25 years of both the sexes were included. Written consent was taken from all subjects and ethical clearance was obtained from the Institutional review Board (IRB; Ref No. CODS/ 2184) of College of Dental Sciences, Davangere, Karnataka according to Rajiv Gandhi University of Health Sciences, Karnataka protocols.

The extracted teeth were required to meet the following inclusion criteria: fully erupted, extracted non-traumatically due to orthodontic reasons, no history of recent periodontal instrumentation or dental prophylaxis, for fluorosed teeth; the fluorotic enamel stains was confirmed by the clinical examination and history of the subjects hailing from natural high water fluoride areas in and around Davangere (fluoride concentration >1.5 ppm). The exclusion criteria were: teeth with proximal caries extending to the cementum, fillings extending beyond cemento-enamel junction (CEJ), and intrinsic stains caused by other reasons such as porphyria, erythroblastosis fetalis, tetracycline therapy, etc.[5] Sample size was 11.72 using  $n = z^2 \sigma^2 / (x_1 - x_2)^2$

## 2.1 Procedural steps

### 2.1.1 Collection of teeth specimens

Healthy nonfluorosed and fluorosed teeth were collected and were immediately washed in running tap water and stored in bottles containing 0.9% saline. [5]

### 2.1.2 Assessment of mineral content of cervical cementum

Teeth specimens were sectioned to 5mm x 5mm thickness using EDS [Energy dispersive spectroscopy Genesis, FEI Quanta 200 high resolution scanning electron microscope] was used to identify the mineral content of cervical cementum. Interaction of an electron beam with a sample target produces a variety of emissions, including x-rays. An energy-dispersive (EDS) detector was used to separate the characteristic x-rays of different elements into an energy spectrum, and EDS system software was used to analyze the energy spectrum in order to determine the abundance of specific elements. EDS was used to find the chemical composition of materials down to a spot size of a few microns, and to create element composition maps over a much broader area. Together, these capabilities provide fundamental compositional information for a wide variety of materials.

The minerals assessed were fluoride, calcium, phosphorous, magnesium, zinc, potassium, carbonate.

### 2.1.3 Statistical analysis

The data obtained from mineral content assessment was entered and data was compiled on MS-excel sheet. It was subjected to statistical analysis using SPSS 17.0. Comparison between groups was done using Unpaired t test. P value <0.05 was considered to be statically significant. NS (p>0.05) = not significant; HS (p<0.001) = Highly significant.

## 3. Results

A total of 24 healthy nonfluorosed and fluorosed orthodontically extracted premolars were collected to assess and compare the mineral content of fluorosed versus non fluorosed cementum using energy dispersive spectroscopy (EDS) (Genesis, FEI Quanta 200 high resolution scanning electron microscope). The results of the study are interpreted in table 1.

The percentage of calcium (61.5±1.33), magnesium (0.54±0.09) and phosphorous (24.14±0.55) was more in nonfluorosed group than in fluorosed group. The percentage of sodium (0.37±0.09), fluoride (0.11±0.10), potassium (0.40±0.15), zinc (2.94±0.91), carbonate (10.74 ±2.56) was more in fluorosed group than in nonfluorosed group.

**Table 1: Mineral content of cervical cementum**

Minerals	Nonfluorosed	Fluorosed	t test	p value
Fluoride	0.09 ± 0.09	0.11 ± 0.10	0.633	0.533 (NS )
Calcium	61.5 ± 1.33	60.73 ± 2.15	- 1.06	0.3 (NS)
Phosphorous	24.14 ± 0.55	23.95 ± 0.47	- 0.9	0.37 ( NS)
Magnesium	0.54 ± 0.09	0.47 ± 0.17	- 1.1	0.26 ( NS)
Sodium	0.21 ± 0.10	0.37 ± 0.09	3.7	0.001 (HS)
Zinc	2.41 ± 1.01	2.94 ± 0.91	1.33	0.195 (NS)
Potassium	0.40 ± 0.08	0.40 ± 0.15	0.162	0.873 (NS )
Carbonate	10.25 ± 1.81	10.74 ± 2.56	0.533	0.599 (NS )

\* p value calculated using unpaired t test (S) = Non significant; (S) = Significant

**4. Discussion**

Various methods to assess mineral content of teeth are, Ash content using chemicals [6-8], EDS and EPMA [9,10]. In the present study, assessment of mineral content was done using energy dispersive spectroscopy [EDS]. In the nonfluorosed teeth (cervical cementum) the percentage of calcium, magnesium, and phosphorous was 60.73±2.15, 0.47±0.17, 23.95±0.47 respectively was higher as compared to the percentage of calcium (60.73±2.15), magnesium (0.47±0.17) and phosphorous (23.95±0.47) of fluorosed teeth. In fluorosed teeth (cervical cementum) percentage of sodium, fluoride, potassium, zinc, carbonate was 0.37±0.09, 0.11±0.10, 0.40±0.15, 2.94±0.91, 10.74 ±2.56 respectively was higher as compared to the percentage of sodium (0.37±0.09), fluoride (0.11±0.10), potassium (0.40±0.15), zinc (2.94±0.91), carbonate (10.74±2.56) of nonfluorosed teeth. Surprisingly sodium level (p= 0.001) was significantly higher in fluorosed group. The possible reason requires to be elucidated. As per the authors knowledge this study presents the difference in mineral content of nonfluorosed and fluorosed teeth for the first time in literature.

The following are the studies on mineral content of cementum. The studies related to the objective of our study is not comparable directly as their subjects age, sex, water fluoride exposure and methodology vary and differ from this study .

Nakata *et al* in 1972, conducted a study on 65 extracted teeth from continuous residents of age 20 to 40 years, in optimal (0.8-1.2 ppm) and high (3.0-5.6 ppm) fluoride areas by chemical analysis to determine the effect of fluoride in the drinking water on fluoride, calcium, phosphorus, magnesium in cervical areas of sound and periodontally diseased cementum. It was concluded that fluoride concentration was 0.363±0.02 at 0.8-1.2 ppm and 0.557±0.10 at 3.0-5.6 ppm which was increased with fluoride exposure. The values for calcium, phosphorus, magnesium were calcium concentration (41.1±0.9 at 0.8-1.2 ppm, 37.5±0.6 at 3.0-5.6 ppm), phosphorus concentration (16.8±0.2 at 0.8-1.2 ppm 17.5±0.2 at 3.0-5.6 ppm) and magnesium concentration

0.67±0.02 mg at 0.8-1.2 ppm, 0.84±0.02 at 3.0-5.6 ppm) respectively. [11]

Kraak *et al* in 1972 conducted a study on 2 mandibular second molars obtained from two cows in each of the four groups which had been fed rations containing 12, 27, 49, and 93 ppm F, respectively investigate the effects of long-term fluoride exposure on the fluoride, citrate, and carbonate content of bovine, coronal cementum using chemical analysis method. Fluoride, citrate, carbonate concentrations ranged from 0.3360 to 0.8420%, 1.259 to 0.577% 3.77±0.07% respectively in coronal cementum. Coronal cementum exhibited a much greater capacity to incorporate fluoride than did enamel or dentin. Citrate concentration in all tissues studied steadily decreased with increasing fluoride concentration up to 49 ppm F in the ration. No relation between fluoride and carbonate was apparent. [12]

Neiders *et al* in 1972 conducted a study to assess the amount of calcium, phosphorous and magnesium in human cementum and subjacent dentin of young permanent teeth using 7 orthodontically extracted maxillary and mandibular premolars. Analysis was done using EPMA and was correlated with SEM. The calcium composition of Mandibular right first premolar was 25.5±1.6, maxillary right first premolar was 26.9±1.5, Mandibular left first premolar was 24.8±0.3, maxillary right first premolar was 24.7±1.7, Mandibular left second premolar was 26.2±2.1. The phosphorous composition of Mandibular right first premolar was 14.0±0.8, maxillary right first premolar was 13.7±0.3, Mandibular left first premolar was 11.2±0.2, maxillary right first premolar was 12.6±0.6, Mandibular left second premolar 13.1±0.5. The magnesium composition of mandibular right first premolar was 0.62±0.03, maxillary right first premolar was 0.61±0.05, mandibular left first premolar was 0.50±0.02, maxillary right first premolar was 0.46±0.07, mandibular left second premolar was 0.54±0.05. It was concluded that the mineral content of the cementum and the granular layer of tomes was significantly lower than that of tubular dentin and the hypermineralized zone .[9]

Kato *et al* in 1997 conducted a study on 28 human permanent anterior teeth from individuals residing

in natural high-fluoride area (West Hartlepool, UK; 1.0-1.3 ppm F in drinking water, WHP) and the other from a non-fluoridated naturally low fluoride area (Leeds, UK; 0.1 ppm F in drinking water, LDS) of age 30 to over 60 years using fluoride electrode to determine the effect of water fluoride concentration on the fluoride profile across the entire thickness of the cementum and root dentine. It was reported that WHP cementum had the strongest fluoride concentration correlation with age in the cervical region of the root ( $r = 0.67$ ,  $P < 0.01$ ). The fluoride content of cementum and root dentine in adult residents was related to fluoride content in drinking water.[13]

While the effect of fluoride on a number of chemical constituents has been documented for bone, but only equivocally demonstrated for enamel and dentin, no data appears to be available on the effect of fluoride on the chemical constituents of human cementum. [11] This paucity of literature led us to the objective of our study.

The correlation of bone fluoride and tooth cementum included the results of McCann and Bullock to calculate the ratios of per cent fluoride in the ash of the femora to that of molar dentin in rats fed either a low-fluoride (1 p.p.m.) or high fluoride (50 p.p.m.) diet. This ratio, derived from the mean fluoride contents reported by McCann and Bullock, was 0.50-0.54. Practically nothing is known of the fluoride content of cementum. Since cementum bears a structural similarity to bone and since the turnover of the mineral elements of cementum, as revealed by radioisotopes, is greater than that of enamel or dentin, special attention was paid to comparisons of the fluoride contents of cementum and bone. [14]

The fluoride content of the ash of a fraction of teeth, believed to be cementum, was found to be higher than that of enamel, dentin, and bone derived from the same individuals. The relationship of fluoride contents of cementum and bones allows a fair accuracy in the calculation of fluoride content of the skeleton from that of the former in a population of older people whose fluoride intake was probably not exceptionally high. The fluoride contents of dentin and bones were less well correlated, and, as is to be expected, the fluoride contents of enamel and bone were poorly related. [14]

Fluoride concentration appears to show a gradient from outer surface to inner. Nakagaki *et al* (1985) showed, however, that the gradients of F concentration from the cementum surface towards dentine were not always even. There were occasional peaks of higher concentration and these fluctuations occasionally gave the gradient a highly recognizable profile. Such profiles appeared similar in all regions of the tooth and tended to be characteristic of all teeth from any one individual, often appearing in several teeth from the same mouth [15]. The reason why these individual patterns of F distribution develop is still not

clear. They might reflect systemic changes during the life of the individual; they could also reflect changes in F exposure. Another factor which might be important, however, is the rate of cemental accretion.

Thus in homogenous cementum fluoride concentration may relate directly to anatomical site, varying inversely with the distance from the tissue surface, but the pattern of fluoride distribution is also markedly influenced by histologic structure. Distribution of fluoride in a cellular cementum was lower than cellular cementum as there appears to be a fundamental relationship between Fluoride distribution and histologica structure; the concentration was low in more recently deposited, cellular tissue that is slower the apposition of mineralized tissue, higher the fluoride concentration. [16]

With regard to fluoride uptake by root tissues; Banting and Stamm found that the mean fluoride concentrations in the outer layers of the tooth root were higher in teeth from a fluoridated area compared with a nonfluoridated area.

Excerpts of various studies on mineral content of root cementum are presented here.

- The overall consistently higher concentrations of fluoride in cervical as compared to apical pools may well be a reflection of the effect on cervical cementum of a relatively greater exposure to the oral environment, as might accrue from topical application too.[11]
- The general increase in fluoride concentration with age and fluoride exposure as noted in the cervical and apical pools of normal cementum for the two older age groups parallels the findings of other investigators for bone [17] and dentin and enamel [18] that is as age advances, the fluoride concentration also increased in bone, dentin and enamel .
- How age can serve as a factor for increasing fluoride concentration is explained. The cementum is thin in relation to age and its apposition has therefore been slow. Fluoride, steadily absorbed throughout life, might have achieved these higher concentrations over the same number of years.[15] As in bone and dentin, the Fluoride concentration of cementum increased with age. The total Fluoride concentration of cementum, correlated well with age.
- These average Fluoride values in cementum are roughly similar to avg. values reported for bone from individuals from a similar age range and living in the same low F district and support the findings of Singer and Armstrong. [14]
- In periodontally diseased cementum, fluoride content was more than normal cementum of the same age group and fluoride exposure. [11]

Various factors influencing fluoride levels in cementum are,

- Fluoride in plaque also influences the cervical cementum. [13]
- The calcium content is found depleted in fluorosed teeth and the tooth matrix becomes demineralized. [19,20] The difference in character of the various tooth surfaces is likely to affect the fluoride uptake that is fluoride distribution was different in enamel, dentin and cementum. Brudevold *et al* [21] postulated the following three stages of the deposition of fluoride in enamel: (1) inclusion of small amounts of fluoride in the crystals during calcification; (2) additional pre-eruptive acquisition of fluoride by the surface of the fully calcified enamel through contact with the tissue fluids; and (3) posteruptive uptake of fluoride by the external enamel from the oral environment. The first two stages of deposition, as mentioned above, apply to dentin and cementum as well as enamel. However, in the continuously growing dentin and cementum, inclusion of fluoride during crystal formation will occur throughout the life of the tooth. Once calcified, dentin and cementum continue to acquire fluoride through contact with tissue fluids. This uptake is probably inversely related to the rate of appositional growth, since the slower growth should prolong the fluoride exposure. That this concept is undoubtedly correct was shown by the low concentrations of fluoride found in the external third of the crown dentin, which is known to form rapidly, and the maximal concentrations found in the slowly growing pulpal dentin of older persons and the slowly forming cementum.[21]
- Climate and water consumption are also variables in exposure as indicated in the literature that the more frequent occurrence of mottled enamel in high-fluoride areas with hot climates than in high-fluoride areas with cold climates is due to differences in water consumption.[21]
- Cemental mineral distribution in a given tooth and contralateral teeth is reported. The concentration of fluoride was highest at or near the cementum surface and decreased towards the interior of the tissue. Concentration and patterns of fluoride distribution was the characteristic of the individual subjects. The distribution pattern of fluoride in the contralateral teeth from the same subject were also fairly similar .[15]

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### 5. Conclusion

In the current study the various mineral content of nonfluorosed and fluorosed cervical cementum revealed the percentage of calcium ( $60.73 \pm 2.15$ ), magnesium ( $0.47 \pm 0.17$ ) and phosphorous ( $23.95 \pm 0.47$ ) was higher in nonfluorosed group than in fluorosed group. The percentage of fluoride ( $0.11 \pm 0.10$ ), sodium ( $0.37 \pm 0.09$ ), zinc ( $2.94 \pm 0.91$ ), potassium ( $0.40 \pm 0.15$ ), carbonate ( $10.74 \pm 2.56$ ) was higher in fluorosed group than in nonfluorosed group. The mineral content will influence the hardness which in turn would affect initiation and progression of dental diseases. Further studies are directed to study the histologic and hardness evaluation of nonfluorosed and fluorosed teeth.

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