Study Regarding the Influence of Dental Fluorosis on Enamel and Dentine Mineralization

SORIN ANDRIAN1*, GIANINA IOVAN1, CLAUDIU TOPOLICEANU1, ANTONIA MOLDOVAN1, SIMONA STOLERIU1
1 „Gr.T.Popa” University of Medicine and Pharmacy, Faculty of Dental Medicine, 16 Universitatii Str., 700115, Iași, Romania

The aim of the study was to establish the differences between calcium and fluoride ions concentration in enamel and dentine of the teeth having dental fluorosis with various degree of severity. In this study were used 40 third molars divided in 4 study groups, each related to a specific degree of fluorosis (TF 1-4). The control group was represented by 10 included third molars unaffected by dental fluorosis. The calcium and fluoride ions concentration in the enamel and dentine samples were assessed using spectrophotometry. Data were statistically analysed using ANOVA and post-hoc Bonferroni tests. The teeth having higher severity of fluorosis (TF 3,4) presented significant lower calcium ions concentration in enamel comparing with healthy teeth and the teeth having TF 2,3,4 presented significant lower calcium concentrations in dentine comparing with healthy teeth.

Keywords: dental fluorosis, free Ca2+ ions, free F– ions, enamel, dentine, spectrophotometry

Dental fluorosis is a malformation caused by the chronically intake of high amount of fluoride during dental formation [1, 2]. The major risk factor is represented by the high fluoride intake [3], but other factors also (genetical susceptibility) have been found to be important in fluorosis ethiology [4]. The prevalence of dental fluorosis increases in the past decades, in fluoridated areas varying between 7,7 and 80,9% and in non-fluoridated areas between 2,9 and 42% [2-5].

Fluorosis lesions were described histopathologically as hipomineralisation lesions placed under a high mineralised enamel surface [6]. Numerous studies have shown that these alterations occur as result of excessive fluoride exposure on amelogenesis [7-9], although high concentrations of fluoride can influence all stages of enamel formation [10, 11]. Calcium content of enamel is lower in fluorotic teeth and dental matrix presents a lower degree of mineralisation [12, 13]. Recent studies did not succeed to establish clear correlations between fluorosis severity and dental tissues mineralisation [14]. The knowledge about the mechanisms implied regarding influence of fluor on dentine structure and mineralization is also limited [15-19].

The aim of this study was to establish the differences between calcium and fluoride concentrations in enamel and dentine for teeth affected by dental fluorosis with various degrees of severity.

Experimental part
In this study were used 40 extracted third molars. After extraction, the teeth were washed with water, dried and assessed by a single observer regarding fluorosis severity accordingly to Thylstrup-Fejerskov classification. The teeths were divided in 4 study groups, each related to a specific degree of fluorosis (TF 1-4). The control group was represented by 10 included third molars unaffected by dental fluorosis. Enamel and dentine were sectioned in small fragments and then immersed for 48 h in diethyl ether and acetone (1/1) and dried with acetone. Powder of enamel and dentine was obtained after incubation the dental fragments at 550oC for 24 h. The powder was weighted and treated with 0.25M perchloric acid. Calcium ions concentrations (mg/g) and fluoride ions concentrations (mg/kg) in enamel and dentine samples were assessed by spectrophotometry using Horiba ABX Pentra 400 analyser. Data were statistically analysed using ANOVA and post-hoc Bonferroni test.

Results and discussions
Mean age of patients was 28.0 (age ranging between 17 and 42). Maxillary third molars represented 65% and mandibular molars represented 35% from extracted teeth. Calcium ions concentration ranged between 233mg/g and 364mg/g in enamel samples and between 162mg/g and 335mg/g in dentine samples. Regarding calcium ions concentration in enamel, decreasing tendency was recorded from healthy teeth (315mg/g) to teeth with severe fluorosis (259 mg/g for fluorosis degree 4). Regarding calcium concentration in dentine, decreasing tendency was recorded from healthy teeth (282mg/g) to teeth with severe fluorosis (259 mg/g for fluorosis degree 4). Calcium ions concentrations in enamel and dentine decreased with the increase of fluorosis severity. At the same degree of fluorosis, calcium concentration in dentine is lower comparing with enamel.

The mean values of fluoride concentrations in enamel increased with the increase of fluorosis severity. In healthy enamel the mean ion concentration in enamel was 137.5 mg/kg. In dentine, the mean fluoride concentration was 10.0 mg/kg.

Table 1

<table>
<thead>
<tr>
<th>TF</th>
<th>ENAMEL</th>
<th>DENTINE</th>
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<tbody>
<tr>
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<td>282±2,52</td>
</tr>
<tr>
<td>1</td>
<td>301±2,84</td>
<td>264±1,83</td>
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<td>3</td>
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<td>213±1,63</td>
</tr>
<tr>
<td>4</td>
<td>259±3,47</td>
<td>193±2,31</td>
</tr>
</tbody>
</table>

* email: sorinandrian@yahoo.com
mg/kg and in enamel of the teeth having TF4 was 269.1 mg/kg. In dentine the mean values of fluoride ions were higher than those in enamel. For healthy teeth, the mean fluoride ions concentration in dentine was 285.5 mg/kg and for teeth having TF4 was 546.5 mg/kg.

Data were statistically analysed using ANOVA and post-hoc Bonferroni test. When comparing calcium ions concentration in enamel samples from healthy teeth (TF0) with calcium concentrations for the teeth having fluorosis degree 3 and 4, these tests show significant statistical differences (table 2).

The calcium ions concentration in dentine concentrations was significant lower in the samples having TF2,3,4 when compare with the samples from the healthy teeth. There were significant statistical differences when compare the fluoride ions concentration in enamel of the teeth with TF0 and TF1 with the teeth having TF3 and TF4. The significant statistical differences were obtained when comparing fluoride concentrations in the enamel samples of healthy teeth (TFI 0) or teeth with fluorosis degree 1 with enamel samples of teeth with fluorosis degree 3 and 4 (TFI 3 and TFI 4). The significant statistical differences were obtained when comparing fluoride concentrations in teeth with fluorosis degree 2 and teeth with fluorosis degree 4 (table 3). Regarding fluoride concentrations in dentine samples, significant statistical differences were obtained when comparing fluoride concentrations in teeth with fluorosis degrees 3 and 4 with fluoride concentrations in teeth with fluorosis degree 1 or teeth unaffected by fluorosis (table 4).

This study demonstrated a decrease of calcium ions concentration in fluorotic teeth comparing with healthy teeth, observation similar to related studies [12]. Shinoda (1983) reported that after a prolonged administration of fluoride, there was a high reduction of calcium and phosphat content in mature enamel because of structural alteration of ameloblastic layer [20]. The explanation for low concentration of calcium ions in fluorotic enamel is related to the influence of fluoride ions in regulation of calcium concentration in the mineralisation environment, resulting in an alteration of calcium-dependent proteases. It is possible that excess of fluor to interfere with the activity of proteases because of decrease in free-calcium concentration [21]. Acute overdoses of fluoride determine initial hypermineralisation, followed by hypomineralisation in enamel and dentine. These responses can be explained by a series of events characterised by the fast increase of fluoride supply (hypermineralisation) and accelerated consume of calcium ions, resulting in a temporary inhibition (hypomineralisation) or delay in mineralization processes [8].

Studies on matrix molecules of fluorotic teeth demonstrated that some sulphate isomers of glycosaminoglycans (dermatan sulphate) accumulate in tissues as a result of fluoride supply. This process is followed by demineralisation of teeth tissues. Demineralised areas from dental structure cannot be remineralised because of the presence of dermatan sulphate in dental organic matrix [13]. The fluoride administration on long term conducts to structural alteration of enamel surface and to adverse effect on biochemical elements.

### Table 2
BONFERRONI MULTIPLE COMPARISONS FOR CALCIUM IONS CONCENTRATIONS IN ENAMEL

<table>
<thead>
<tr>
<th>(j) SF</th>
<th>(i) SF</th>
<th>Mean Difference (i-j)</th>
<th>Std. Error</th>
<th>p.</th>
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<td>1.46545</td>
<td>1.000</td>
</tr>
<tr>
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<td>0.090</td>
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<tr>
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<td>0.004</td>
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<td>1.000</td>
</tr>
<tr>
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<tr>
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</tr>
<tr>
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<td>0.000</td>
</tr>
<tr>
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<td>0.000</td>
</tr>
<tr>
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<td>0.000</td>
</tr>
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<td>0.000</td>
</tr>
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</table>

* The mean difference is significant at the 0.05 level.

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Conclusions

Teeth with higher degree of dental fluorosis (3 and 4) present lower calcium ions and higher fluoride ions concentrations when compared to teeth with less severe fluorosis and healthy teeth, both for enamel and dentine.

Knowledge of the mineral structure of fluorotic teeth is mandatory for further understanding of the response of these teeth to oral challenges including caries, as well as for implementation of specific strategies for restorative treatment.
References
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Manuscript received: 19.04.2012