SALIVA CHARACTERISTICS OF CHILDREN WITH DENTAL FLUOROSIS AND THE EFFECT OF HIGH FLUORIDE WATER ON THE SALIVA

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SUMMARY: The aim of this study was to evaluate salivary characteristics, including salivary fluoride (F), calcium (Ca), phosphorus (P), protein, amylase, lysozyme, and lactoferrin levels, in 60 children (selected from 420 examined) who were divided into three groups: (1) children with dental fluorosis, (2) children without dental fluorosis and healthy teeth, but no caries, and both groups living in Isparta, Turkey with high F (2.7–2.8 ppm) water, and (3) children without dental fluorosis and healthy teeth living in the nearby city of Afyon with low F (0.153 ppm) water. No statistical differences between groups were found except for lysozyme levels. Further studies on the effect of different water F levels on salivary composition are considered desirable.

Keywords: Afyon, Turkey; Dental fluorosis; Fluoride in water; Isparta, Turkey; Lactoferrin; Lysozyme; Saliva characterization.

INTRODUCTION

Dental fluorosis is a hypomineralization of tooth enamel characterized by increased surface and subsurface porosity of the tooth, which causes opacity, pitting, and/or staining of the enamel. The severity of fluorosis depends on the climate and altitude where the individual lives, the water fluoride (F) concentration in that area, additional F sources, F metabolism of the individual, and various genetic factors. Spring waters around volcanic areas may contain high levels of F. The city of Isparta is a volcanic and an endemic fluorosis area in southwestern Turkey. Isparta’s two main water sources are Gölcük Crater Lake and Andaş River (1.55–2.96 mg/L). Human saliva contains components that can influence the composition of oral microflora and the function of microorganisms. Many studies on saliva and dental caries have been reported, but relatively few have been concerned with the relationship between dental fluorosis and saliva contents. Among numerous studies on saliva content, only a few have examined the salivary features of children with dental fluorosis. The aim of this study was to determine the salivary F, Ca, P, protein, amylase, lysozyme, and lactoferrin levels of children with dental fluorosis and to compare them with the salivary content of children with non-fluorosed teeth. In addition, the present study evaluated the effects of high-fluoride water on various contents of the saliva.

MATERIALS AND METHODS

Study Groups: After obtaining the approval of the institutional ethical committee as well as informed consent from the participants, the researchers performed a pilot study to determine which children would participate in the study. A total of
420 children from two different areas in Turkey were examined. The criteria for inclusion were that the children: (a) should be healthy and free of systemic disease, (b) should not have consumed any medications for at least 15 days preceding the saliva collection, (c) should have fully erupted first permanent molars, (d) and should have no cavities. According to these criteria, 60 healthy, 8–10 year-old (8.9±0.68) children were randomly selected and then divided into three groups of 20. Group A children included those who had dental fluorosis rated at severity score of 4 on the Thylstrup-Fejerskov Index (TFI) and were living in Isparta with 2.7–2.8 ppm F in the drinking water. (A TFI dental fluorosis score of 4 indicates marked enamel opacity, including chalky white, but without observable loss of enamel.) Group B children did not have dental fluorosis and had lived in Isparta for no more than 2 years. Group C children had no dental fluorosis and lived in the nearby city of Afyon, which has a low level of F (0.153 ppm) in its drinking water.

**Collection of the Saliva Samples:** A special diet containing cheese and bread was given to the participants for breakfast. The saliva was collected in the morning to minimize the effects of circadian rhythm. The unstimulated saliva (3 mL) and stimulated saliva (4 mL) were collected into sterilized polyethylene cups. The stimulated saliva was used for F analysis.

**Analysis of the Saliva Samples:** For the determination of saliva F levels, an ion-specific electrode (Mettler-Toledo GmbH, AG, Switzerland), a pH meter (Mettler-Toledo MA235 pH/Ion Analyzer, Switzerland) and a pH electrode (Mettler-Toledo In Lab 302, Mettler-Toledo AG, Undurf, Switzerland) were used. The F levels were measured as mV and then transformed into “ppm.” The salivary Ca and P levels and amylase activity were determined by atomic absorption spectrophotometry. The salivary protein level was determined with the Sigma Total Protein Kit, Micro Lowry, Peterson’s Modification (Sigma-Aldrich Inc., Missouri, USA). The salivary lactoferrin and lysozyme levels were evaluated with AIDA Lactoferrin and Lysozyme kits (Autoimmune diagnostic assays, AIDA GmbH, Germany).

**Statistical Analysis:** Results were analyzed statistically through the Mann-Whitney U test and the Student T-test. All analyses were performed using the SPSS statistical package for Windows, version 15.0 (SPSS, Chicago, IL, USA). Significance was set at p<0.05.

**RESULTS AND DISCUSSION**

Table 1 shows the salivary F, Ca, P, protein, amylase, lysozyme, and lactoferrin levels of children with and without dental fluorosis but no dental caries. The only statistically significant difference was found in the lysozyme levels (p = 0.038). No statistically significant differences between males and females were observed.
There are a limited number of papers available that evaluate the salivary parameters of children with fluorosis, so comparisons were made between control groups of other studies that tested the same age group.

Previous studies report that the average salivary F concentration of healthy individuals is 0.01–0.04 ppm. Alamoudi et al. have reported much higher salivary F concentrations during the mixed dentition period: 0.145±0.006 ppm. Another study reported that the salivary F concentrations of children living in low (0.1 ppm) and high (1.2 ppm) F areas is 0.3 and 0.9 µM/L (0.0057 and 0.017 ppm), respectively. Martin-Gomez et al. reported that there was no statistical difference between the saliva concentrations of children with fluorosis and the control group, but they did find that the resting saliva F concentrations were higher in the children with fluorosis.

The present study also found no statistically significant difference between salivary F levels of the different groups. The F levels did not differ possibly because the saliva interacts with enamel, plaque, and plaque fluid. A study comparing adolescents with and without dental fluorosis also revealed no statistically significant difference between salivary F, Ca, and phosphorus levels. Our values for salivary F concentration in children are higher than the levels reported in the study carried out in adolescents. This age difference may be an experimental artifact, or the difference may be linked to the fact that we measured the parameters only of children with no cavities.

It has previously been reported that salivary lysozyme and lactoferrin concentrations decrease with age. Another study found that salivary lysozyme and lactoferrin concentrations are low in infants. While some studies point to an increase in the activity of amylase and total protein concentration with age, other research shows no relationship between them. Some research has claimed that F ion concentration has an inhibitory effect on lysozyme activation, but other work has shown that the pH of the medium is more important. In our study, the

<p>| Table 1. Mean values of salivary parameters for the three groups of 8–10 year-old children |
|---------------------------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Group A (n=20)</th>
<th>Group B (n=20)</th>
<th>Group C (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F (ppm)</td>
<td>0.05±0.002</td>
<td>0.06 ±0.02</td>
<td>0.06 ±0.076</td>
</tr>
<tr>
<td>Lactoferrin (U/mL)</td>
<td>4.03 ±2.4</td>
<td>2.7 ±2.1</td>
<td>4.4 ±2.5</td>
</tr>
<tr>
<td>Lysozyme (U/mL)</td>
<td>1.7±2.5</td>
<td>0.2±0.09*</td>
<td>2.2±3.4</td>
</tr>
<tr>
<td>Amylase (U/mL)</td>
<td>104729.8±62543.4</td>
<td>72095.85±50181.6</td>
<td>96674.9±47411.7</td>
</tr>
<tr>
<td>Protein (mg/dL)</td>
<td>158.7±56.4</td>
<td>138.5±57.1</td>
<td>164.2±59.4</td>
</tr>
<tr>
<td>Ca (mg/dL)</td>
<td>3.3±0.7</td>
<td>3.2±0.9</td>
<td>3.5±0.7</td>
</tr>
<tr>
<td>P (mg/dL)</td>
<td>15.0±3.3</td>
<td>16.4±3.1</td>
<td>14.8±4.0</td>
</tr>
</tbody>
</table>

* p < 0.05
Group B children lysozyme levels were much lower than the lysozyme levels of the other two groups. We found no relation between salivary lysozyme concentration and the F level of the drinking water. The F ion was supersaturated within the tooth structure of Group A (the children with fluorosis). In Group B, the teeth were non-fluorosed with only the high F content of drinking water acting as a local F application. Gingival inflammation around the exfoliating deciduous and erupting permanent teeth, the number of teeth existing in the oral cavity, and the extent of eruption during mixed dentition may have influenced the lysozyme levels of Group B. It is unknown, however, to what extent these parameters may have differed among the three groups of children. Liu et al. evaluated the effects of genetic factors on the fluorosis mechanism and found a relationship between gene susceptibility and tolerance to dental fluorosis. Other reports have also revealed a relationship between genetic influences and fluorosis. To help explain the difference in lysozyme levels between the three groups in the present study, a genetic study might prove useful.

In conclusion, further studies of how different water F levels might affect salivary composition are still needed.

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REFERENCES

Effect of high fluoride water on saliva of children with dental fluorosis

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