Fracture reattachment in an immature permanent incisor with talon's cusp. A rare case report
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Does the chemistry in the saliva of Down syndrome children explain their low caries prevalence?

**ABSTRACT**

**Aim** The present study focuses on the relationship between dental caries and saliva components such as phosphate, calcium, potassium, chloride as well as α-amylase in children with Down syndrome.

**Materials and Methods** Forty-five Caucasian sibling pairs, with the mean age of 13±4 years compose the final sample. Stimulated whole saliva was collected from DS children and their siblings and an automatic analyser quantified the biochemical parameters.

**Results** Down syndrome children presented lower caries rates. The salivary concentration of calcium, phosphate, potassium and chloride did not differ between DS and sibling children. In respect to α-amylases, the absolute salivary concentration as well as salivary secretion rate was similar between DS and sibling controls.

**Conclusion** In conclusion, no correlation between dental caries and salivary ionic composition as well as α-amylase secretion rate was found in DS children.

**Keywords** Caries, Down syndrome, Ions, Paediatrics, Saliva

**Introduction**

Down's syndrome (DS) is a genetic disorder resulting from a trisomy of chromosome 21 that leads to multiple abnormalities. Systemic manifestations in DS are common, such as recurrent respiratory infections, congenital heart defects, immunologic disorders, and hypothyroidism [Loiseau and Nardoux, 1976; Vigild, 1986; Desai, 1997]. In respect to oral health, DS individuals present smaller palate and maxilla when compared to the mandible, delayed eruption of deciduous and permanent dentitions as well as agenesis of teeth [Loiseau and Nardoux, 1976; Vigild, 1986; Desai, 1997; Shore et al., 2010]. In addition, it was shown that DS individuals have increased prevalence of periodontal disease, which develops at early age and is rapidly progressive [Reuland-Bosma and van Dijk, 1986; Shapiro et al., 1969], but have lower prevalence of dental caries [Areias et al., 2011; Cogulu et al., 2006; Fung and Allison, 2005; Lee et al., 2004; Stabholz et al., 1991]. In DS individuals, the lower prevalence of dental caries may be caused by different environmental factors, congenital oligodontia, and delayed eruption as well as by altered salivary composition [Shore et al., 2010; Lenander-Lumikari and Loimaranta, 2000; Petersen, 2003; Cornejo et al., 2008; Latner, 1983; Krol, 2004; Creighton, 1998; Fung and Lawrence, 2008; Hennequin et al., 2000, Areias et al., 2008].

It is generally accepted that saliva secretion and salivary components secreted in saliva are important for oral health [Lenander-Lumikari and Loimaranta, 2000]. Saliva not only lubricates the oral tissues, making oral functions such as speaking, eating, and swallowing possible, but also protects teeth and oral mucosa surfaces, in different ways [Lenander-Lumikari and Loimaranta, 2000; Shapiro et al., 1991; Siqueira et al., 2004; Siqueira and Nicolau, 2002], playing a critical role in the prevention or reversal of the caries process. Saliva provides calcium, phosphate and proteins that maintain super saturation of calcium in the plaque fluid, proteins and lipids that form a protective film on the surface of the tooth, antibacterial substances and buffers [Kavanagh and Svehla, 1998; Vijayaprasad et al., 2010; Siqueira et al., 2007; Davidovich et al., 2010]. The salivary components neutralise the acids produced by bacterial metabolism in the plaque, raise the pH and reverse the diffusion gradient for calcium and phosphate [Kavanagh and Svehla, 1998; Vijayaprasad et al., 2010; Siqueira et al., 2007; Davidovich et al., 2010]. Thereby, they return calcium and phosphate to the subsurface lesion, where these ions can regroup new surfaces on the crystal remnants that were produced by demineralisation. These so-called “remineralised” crystals have a veneer of much less soluble mineral. Saliva also clears carbohydrates and acids from the plaque [Siqueira, 2005; Featherstone, 2000; Okahashi et al., 2011].

The salivary amylase is an important enzyme present in the oral cavity. Its main function is to promote the hydrolysis of starch turning it into maltose. This could serve as a substrate for oral bacteria inducing the production of acids, which can promote enamel demineralization [Lenander-Lumikari and Loimaranta,
Material and methods

All DS children with age between 6 and 18 years included in a national database were invited to participate in the present study. Controls were sibling-matched, closest in age. The final sample was constituted by 45 Caucasian sibling pairs. Informed consent was obtained from the participants' parents, who were provided with detailed information on the study protocol. The Ethics Committee of the Faculty of Dental Medicine of Porto University approved both the consent form and the research protocol (study approved with number of the opinion to approve 00005). It complied with the rules of conduct expressed in the Declaration of Helsinki (2000) and national legislation guaranteeing the necessary confidentiality of personal information collected.

None of the children had systemic diseases and did not take any medication for at least 3 weeks before saliva collection.

Dental caries examinations were carried out using a mirror and explorer in accordance with the World Health Organization criteria and methods. The total number of decayed, missing and filled primary and permanent teeth (dmft, DMFT) were recorded for each participant. The mean age of sibling contrais was 12.8±3.7 years old and included 60% males. The DMFT/dmft score of the DS group in comparison to their siblings was 1.02±2.42 vs. 1.84±3.13, where decayed component (D) was 0.44±1.27 vs. 0.87±2.12, missing teeth (M) was 0.42±1.78 vs. 0.93±1.64 and filled teeth (F) was 0.16±0.67 vs. 0.04±0.21. The rate of caries-free children was higher in DS group than in sibling group (Fig. 1).

The salivary concentration of calcium, phosphate, and chloride were evaluated by potentiometry using ion selective electrodes. Phosphate was detected by UV using phosphomolybdate whereas calcium was determined by a photometric test, using ortho-cresolphthalein complexone. In addition, α-amylases were detected by an enzymatic photometric test, using as substrate 4,6-ethylidene-(G7)-p-nitrophenyl-(G1)-α-D-maltoheptaoside (EPS-G7). The α-amylases secretion rates (U/min) were calculated by multiplying α-amylases absolute levels with salivary flow [Featherstone, 2000].

Statistical analysis

The categorical variables were described through relative frequencies (%), whereas continuous variables were described using mean ± standard deviation (SD). Were applied when appropriate Chi-square independence test to analyse hypotheses regarding the categorical variables and Student t-test concerning continuous variables. It was considered a significance level of 0.05. The analysis was performed using the statistical analysis program Statistical Package for Social Sciences v.17.0 (SPSS, Chicago, IL, USA).

Results

The mean age of the DS children was 12.7±4.0 years old and included 49% males whereas the mean age of sibling controls was 12.8±3.7 years old and included 60% males. The DMFT/dmft score of the DS group in comparison to their siblings was 1.02±2.42 vs. 1.84±3.13, where decayed component (D) was 0.44±1.27 vs. 0.87±2.12, missing teeth (M) was 0.42±1.78 vs. 0.93±1.64 and filled teeth (F) was 0.16±0.67 vs. 0.04±0.21. The rate of caries-free children was higher in DS group than in sibling group (Fig. 1).

The salivary concentration of calcium, phosphate,
SALIVA OF DOWN CHILDREN AND THEIR CARIES PREVALENCE

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<tr>
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<th>C</th>
<th>DS</th>
<th>P*</th>
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<tbody>
<tr>
<td>Phosphate, mg/dL</td>
<td>12.8±6.0</td>
<td>11.0±5.7</td>
<td>0.196</td>
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<tr>
<td>Calcium, mg/dL</td>
<td>4.26±1.13</td>
<td>3.44±2.27</td>
<td>0.133</td>
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<tr>
<td>Potassium, mmol/L</td>
<td>17.1±6.7</td>
<td>15.8±6.7</td>
<td>0.416</td>
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<tr>
<td>Chloride, mmol/L</td>
<td>22.3±4.6</td>
<td>20.5±5.1</td>
<td>0.112</td>
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<tr>
<td>α-Amylases, U/L</td>
<td>360±487</td>
<td>516±668</td>
<td>0.212</td>
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<tr>
<td>α-Amylases secretion rate, U/min</td>
<td>181±281</td>
<td>244±478</td>
<td>0.450</td>
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Values are means±SD. *p values were calculated using Student's t-test.

potassium and chloride did not differ between DS and sibling children (Table 1). In respect to α-amylases, the absolute salivary concentration as well as salivary secretion rate was similar between DS and sibling controls (Table 1).

Discussion

The present study was undertaken with the aim of understanding the factors involved in the reduced dental caries prevalence in DS children. In comparison to their siblings, the DS children showed no differences in salivary concentration of calcium, phosphate, potassium and chloride as well as the α-amylase secretion rate.

Although caries is an infectious disease, its severity and progression are regulated by several factors. Recent studies have attributed to saliva a large number of functions mediated by both the inorganic and organic components that should be considered in the assessment of the role of human saliva on dental caries development [Lenander-Lumikari and Loimaranta, 2000; Cornejo et al., 2008; Okahashi et al., 2011]. In terms of ionic composition the ions in saliva which contain the most are calcium and phosphate. Because they will help to prevent dissolution of dental enamel [Lenander-Lumikari and Loimaranta, 2000; Kavanagh and Svehla, 1998; Vijayaprasad et al., 2010; Siqueira, 2005, Featherstone, 2000; Okahashi et al., 2011]. The other ions in saliva are of interest more for their role in the secretion of saliva than for their activity in the oral cavity [Lenander-Lumikari and Loimaranta, 2000].

In terms of ionic saliva composition, DS children presented no differences in comparison to their siblings. Other researchers reported the same result for phosphate [Siqueira et al., 2004; Davidovich et al., 2010], however, there are conflicting results in the case of calcium, potassium and chloride levels of individuals with DS. Several authors examined salivary calcium concentration in DS population and most of them did not find any differences between DS and non-DS populations [Siqueira et al., 2004; Siqueira et al., 2007; Okahashi et al., 2011]; however, a recent report showed higher levels of salivary calcium concentrations in DS children [Davidovich et al., 2010]. In relation to potassium concentration in saliva some reports found higher concentrations in DS saliva [Davidovich et al., 2010; Siqueira, 2005; Okahashi et al., 2011] whereas others found reduced levels in comparison to controls [Creighton, 1998]. In relation to chloride concentration in saliva different researchers found no differences [Okahashi et al., 2011] higher [Davidovich et al., 2010] or lower [Siqueira, 2005] salivary concentration in DS individuals. These differences observed in salivary sialochemistry may be related to different saliva collection techniques: stimulated or non-stimulated, whole or direct from salivary glands.

Other reasons that may justify the conflicting results between research groups may be related to the control group used. In our study, the control group was healthy age-matched siblings in order to minimize the influence of environmental factors such as diet and oral hygiene habits as well as familial predisposition. However, most of the studies referred used an unrelated healthy group as controls.

In addition to salivary ions, salivary α-amylases were also studied in DS children. Given that this enzyme is produced primarily by the parotid glands, its activity has been proposed as a marker of parotid gland development [Siqueira, 2005]. Siqueira and co-workers found reduced levels of α-amylases in DS children younger than 10 years old [Siqueira et al., 2002; Siqueira et al., 2007], suggesting parotid glands delayed development in DS children. However, in our study the absolute salivary concentration as well as the salivary secretion rate of α-amylases did not differ between control and DS children with mean age of 13 years old. This result may suggest that after 10 years old, the parotid glands of DS children may be fully developed. This fact may also contribute to the absence of differences in ionic saliva composition between DS and their siblings.

Another important factor that may influence caries lesions development is children’s flow rate. Regarding DS individuals, most researchers [Areias et al., 2012; Chaushu et al., 2007; Siqueira et al., 2004], though not...
all [Cogulu et al., 2006], reported reductions in salivary flow rate. The lower salivary flow of DS children could in theory increase their caries susceptibility [Takahashi et al., 2011; Tenovuo et al., 1997], so, other factors are clearly favoring these children in a caries-protective way. Further research is suggested perhaps in relation to the quantity of resting and stimulated saliva compared with the controls.

Additionally, infrequent snacking of DS children may also be what distinguishes them from the control group given that healthy controls may buy candy or snacks in an autonomous way. However, in a previous study we did not found differences regarding the consumption of sweet or acidic food between DS children or their siblings [Areias et al., 2012].

Conclusion

In conclusion, no correlation between dental caries and salivary ionic composition as well as α-amylase secretion rate was found in DS children.

Acknowledgement

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Conflict of interest statement

The authors of this study declare that they do not have any commercial or associative interest that represents a conflict of interest in connection with this work.

References