A comparison of the sialochemistry, oral pH, and oral health status of down syndrome children to healthy children

ESTI DAVIDOVICH[1], DORON JAACOB AFRAMIAN[2], JOSEF SHAPIRA[3] & BENJAMIN PERETZ[4]

[1]Department of Paediatric Dentistry, Hebrew University, Hadassah Faculty of Dental Medicine, Jerusalem, Israel, [2]Head of Salivary Gland Clinic and Saliva Diagnostics Laboratory, Department of Oral Medicine, Hebrew University-Hadassah School of Dental Medicine Jerusalem, Israel, [3]Department of Paediatric Dentistry, Hebrew University, Hadassah Faculty of Dental Medicine, Jerusalem, Israel, and [4]Department of Paediatric Dentistry, The Maurice and Gabriela Goldschleger School of Dental Medicine, Tel Aviv University, Tel Aviv, Israel

Background: The aetiology of low caries incidence in Down syndrome (DS) children is not entirely clear.

Aim. To compare sialochemistry and oral mucosal pH between Down syndrome children with caries (DS-Ca) and caries free (DS-CaF), and healthy children with caries (C-Ca) and caries free (C-CaF).

Design. The study group comprised 70 children with DS (mean age 4.41 ± 1.9 years); 32 healthy children (mean age 9.22 ± 2.7 years) served as control. Groups were further subdivided according to caries status: DS-Ca, DS-CaF, C-Ca and C-CaF. Sialochemistry analysis included calcium (Ca), sodium (Na), potassium (K), and chloride (Cl). Mucosal pH, plaque and gingival indices (PI and GI), and caries status were recorded.

Results. DMFT/dmft were significantly lower in the DS group. Cl and Ca levels were significantly higher in the DS-Ca compared to the C-Ca and the C-CaF children. Na and K were significantly higher in DS-Ca group compared to DS-CaF group. PI and GI were significantly higher in DS-C children compared to DS-CaF children.

Conclusions. DS may manifest itself in the salivary glands. Consequently, different electrolyte salivary environment may form, leading to lower caries rates among DS children.

Introduction

Down syndrome (DS) is a genetic disorder caused by a trisomy of chromosome 21 with an incidence of 1: 800 to 1:1000 births. The syndrome is characterized mainly by mental retardation, cardiovascular, hematopoietic, musculoskeletal and nervous system anomalies, as well as other phenotypic abnormalities[1,2].

Numerous oral manifestations have been described in DS individuals including low incidence of dental caries, high incidence of periodontal diseases, mouth breathing resulting in dry mouth, fissured tongue and lips, high incidence of mucosal ulcers, candidiasis and acute necrotizing ulcerative gingivitis compared to healthy individuals. Patients with DS also demonstrate macrologlossia, imbalanced occlusal and soft tissues forces, open bite, impaired chewing and consequent difficulty in self cleansing of teeth[2,3].

One of the most prominent oral manifestations in DS patients is low incidence of dental caries. Stabholtz *et al.* found that among children with DS, 84% were caries-free[4]. This low caries incidence in children with DS is in spite of the presence of increased risk factors for caries such as cariogenic diet, decreased salivary flow rate, mouth breathing, imbalanced occlusal forces and poor access to oral hygiene[2,4–16]. Several studies addressed the aetiology of the low incidence of caries, yet the exact pathogenesis is unclear.

Saliva has numerous beneficial actions: it protects the hard and soft tissues from desiccation, enhances taste, lubricates the food bolus, and facilitates chewing and swallowing. Saliva also assists in clearance of food debris and serves as a buffering system. Saliva's

Correspondence to:
Esti Davidovich, Department of Paediatric Dentistry, The Hebrew University Hadassah School of Dental Medicine, PO Box 12272, Jerusalem, 91120 Israel.
E-mail: dr-st@012.net.il

buffering capacity is carried out by three different mechanisms in order to maintain non acidic oral pH balance[16–18]. Salivary pH changes with age, and pH levels in infants are lower than in adults[19].

One of the hypotheses suggested in the literature for the low caries incidence in DS individuals focuses on their higher pH level in the oral cavity and their irregular salivary buffering capacity. While some studies showed better salivary buffering capacity in DS individuals[6,14], others found no such differences, or even decreased buffering capacity in DS[2,7,12,20].

The reason for these conflicting results may be attributed to different techniques of measurements. Some investigators examined pH levels by using indicator papers while others have used digital methods. Also, there were differences in the sites of measurements, where some measured pH levels extra-orally in a tube, and others did it directly on the floor of the mouth or in other intra-oral site[2,7,12,13,20,21]. Siqueira *et al.*[13] suggested that there was an alteration in the secretory pathways of the duct and/or the acinar cells of salivary glands among DS children, due to differences in salivary electrolytes levels[13]. The authors also found differences in the concentrations of sodium (Na) and potassium (K) while no differences were found for phosphorus (P), zinc (Zn), magnesium (Mg), and calcium (Ca) compared to healthy children. They hypothesized that these alterations in acinar cells metabolism could have led to low caries incidence among this population[13].

The purpose of the present study was to better understand the mechanisms of the differences between children with DS and healthy children with regard to caries by investigating the oral health status, oral mucosal pH and the sialochemistry (concentration of salivary ions) in these populations.

Materials and methods

Upon approval by the Institutional Review Board for Research on Human Subjects and informed consent, 70 children with DS were examined. Thirty-two healthy children who attended the Department of Paediatric Den-

tistry at the Hebrew University Hadassah School of Dental Medicine for routine examinations or treatment were randomly selected as a control group (C).

Based of their caries status, all children in each group were subdivided as follows:
I. Caries free group (DS-CaF and C-CaF).
II. Caries group (DS-Ca and C-Ca).

Evaluations of the clinical oral variables were carried out by two authors (ED and BP), and diagnosis and scores were made once consensus was reached.

The clinical and laboratory variables were the following:
1) Decayed, missing or filled teeth (DMFT/dmft), according to the WHO guidelines: using a mirror and a blunt probe with natural light[23].
2) Plaque index (PI)[24] and gingival index (GI)[25] as follows: on the buccal and lingual surfaces of the maxillary first permanent or the right primary second molar; the maxillary primary or permanent left central incisor, the maxillary right first bicuspid or primary molar, the mandibular left first permanent or second primary molar, the right mandibular central primary or permanent incisor; the mandibular right first bicuspid or first primary molar.
3) Oral mucosal pH measurements: the mucosal pH was measured with a flat, glass electrode pH meter (HANNA instruments HI 8424, Padova, Italy). Two sets of measurements were collected from four locations: the hard palate, the right and left buccal mucosa and the middle tongue. This order was kept in all measurements[22].
4) Saliva analysis: unstimulated whole saliva was collected from the floor of the mouth near the Wharton's ducts orifices[25–27]. The children were sitting on their care givers' laps and saliva was sucked with 2 mm syringes from the floor of the mouth. Older children were asked to spit into a tube. Saliva was collected in a quiet room between 8:00 to 12:00 AM, at least 1 h after eating, tooth brushing or mouth wash. Saliva samples were kept at −80°C until analysis was obtained. Thereafter, saliva samples were thawed, then

centrifuged (4000 rpm, 20 min, 25°C). The supernatants were analysed using a Hitachi 917 autoanalayzer and the concentrations of Na, K, Cl, Ca were measured (Roche Ltd, Basel, Switzerland)[28].

The patients and their parents, were informed about the oral treatment needs of the children, and were offered comprehensive oral treatments after the examinations.

Statistical analysis

Chi-square and Fisher–Irwin tests were used to analyse the categorical variables. Due to the large variability of the salivary component, the Kruskal–Wallis test was used to compare between the subgroups. The results of the pH scores between the subgroups were compared by one-way analysis-of-variance (ANOVA). The correlation between pairs of parameter levels were analysed by the Pearson correlation.

Results

The DS-Ca group included 20 (65%) males and 11 (35%) females. Their mean age was

6.01 ± 1.61 years (range 2.16–9 years). The DS-CaF group included 24 (62%) males and 15 (38%) females whose mean age was 2.5 ± 1.18 years (range 1–6 years) (Table 1).

Caries status

The mean DMFT/dmft score in the DS group was significantly lower than in the C group (3.37 ± 0.56 and 5.9 ± 0.80 respectively, $P = 0.012$). Table 1 shows the gender and age distributions of the caries status among the DS and the C groups. In the DS group, 39 children (56%) were caries free and 31 (44%) demonstrated caries, while in the C group 7 children (22%) were caries free and 25 children (78%) had caries. No significant gender differences were noted.

Oral mucosal pH

Table 2 shows the mean pH scores from the four intraoral mucosal sites (the hard palate, the right and left buccal mucosa and the middle tongue). The mean pH score in the DS-Ca group (6.19 ± 0.36) was significantly lower

Table 1. Caries status among the DS and the C groups.

	Down syndrome group (DS)		Control group (C)	
N	70		32	
Male (%)/female (%)	44 (63)/26 (37)		23 (72)/9 (28)	
Age (*Y*); mean ± SD, [range]	4.41 ± 1.97 [1–9]		9.22 ± 2.74 [5–15]	
	Caries (DS-C)	**Caries free (DS-CF)**	**Caries (C-C)**	**Caries free (DS-CF)**
N (%)	31(44)	39(56)	25 (78)	7(22)
Age (*Y*); mean ± SD, [range]	6.01 ± 1.6 [2.16–9]	2.5 ± 1.18 [1–6]	8.9 ± 2.7 [5–14]	10.2 ± 3 [6–15]
Male (%)/female (%)	20(65)/11(35)	24(62)/15(38)	19(76)/6(24)	4(57)/3(43)

One-way ANOVA.

Table 2. Mean pH scores from four mucosal sites in the DS and in the C groups.

pH score (mean ± SD, [range])	Down syndrome group (Ds)		Control group (C)	
	Caries free (DS-C)	**Caries (Ds-CF)**	**Caries free (C-C)**	**Caries (C-CF)**
Average all sites pH	6.19 ± 0.36 [5.32–7.15]	6.43 ± 0.32 [5.67–7.25]*	6.65 ± 0.36 [5.90–7.32]**	6.73 ± 0.12 [6.62–6.98]*
Hard palate	6.46 ± 0.58 [5.5–7.99]	6.58 ± 0.34 [5.84–7.18]	7.14 ± 0.78 [5.92–9.18]**^^	7.32 ± 0.51 [6.72–8.09]**^^
Right mucosa	6.12 ± 0.41 [5.24–7.11]	6.39 ± 0.41 [5.47–7.38]*	6.43 ± 0.33 [5.76–7.03]*	6.56 ± 0.44 [6.21–7.43]*
Left mucosa	6.06 ± 0.39 [5.25–7.03]	6.38 ± 0.40 [5.44–7.20]**	6.39 ± 0.43 [5.23–7.37]*	6.46 ± 0.14 [6.31–6.72]*
Tongue	6.11 ± 0.39 [5.12–7.06]	6.37 ± 0.44 [5.49–7.28]*	6.65 ± 0.39 [5.73–7.44]**^	6.59 ± 0.21 [6.25–6.89]*

*Significant difference from DS-Ca, ^Significant difference from DS-CaF, •Significant difference from C-Ca, ×Significant difference from C-CaF. *$P < 0.05$, **$P < 0.01$ (one-way ANOVA).

than the scores of the DS-CaF (6.43 ± 0.32), of the C-Ca (6.65 ± 0.56) and of the C-CaF groups (6.73 ± 0.56) (P = 0.025, 0.0001, and 0.001 respectively).

Sialochemistry

Figure 1 demonstrates the concentrations of Cl, Ca, Na, K in the saliva.

Chloride (Cl). The Cl concentration in the DS-Ca group (mean = 23.7, median = 22.20 ± 11.5 mg/dL) was significantly higher than in the C-Ca (mean = 15.7, median = 14.50 ± 4.8 mg/dL) and the C-CaF groups (mean = 16.17, median = 14.00 ± 4.96 mg/dL) (P = 0.001 and 0.048 respectively).

Calcium (Ca). The Ca concentration in the DS-Ca (mean = 7.67, median = 5.65 ± 4.61 mg/dL) was significantly higher than in the DS-CaF (mean = 4.36, median = 4.00 ± 2.48 mg/dL), the C-Ca (mean = 1.89, median = 1.60 ± 1.11 mg/dL) and the C-CaF groups (mean = 1.62, median = 1.55 ± 0.32 mg/dL) (P = 0.012, 0.0001, and 0.001, respectively).

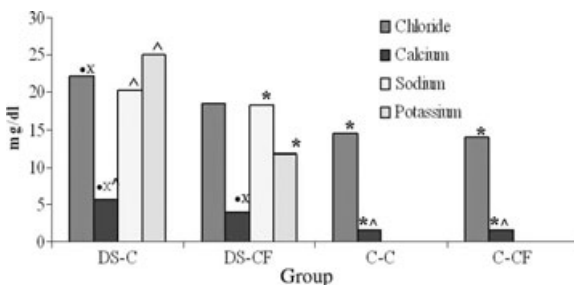Ca level in the DS-CaF group was also significantly higher than in the C-C and the

Fig. 1. Sialochemistry results in all examined groups. *Significant difference from DS-Ca, ^Significant difference from DS-CaF •Significant difference from C-Ca, ˣSignificant difference from C-CaF.

C-CF groups (P = 0.002 and 0.01, respectively) (Fig. 1).

Sodium (Na) and potassium (K). The DS-Ca group demonstrated significantly higher concentrations of Na and K (mean = 23.14, median = 20.2 ± 8.46, and mean = 25.3, 24.96 ± 6.23 mg/dL) than the DS-CaF group (mean = 19.0, median = 18.3 ± 13.72, and mean = 17.97, 18.44 ± 11.78 mg/dL) (P = 0.05 and 0.025, respectively) (Fig. 1).

Plaque and gingival indices (PI and GI)

The DS-Ca group presented significant higher mean PI score than the DS-CF group (1.46 ± 0.55 and 1.04 ± 0.65, respectively; P = 0.028).

The mean GI score in the DS-Ca group was significantly higher than in the C-Ca group (1.29 ± 0.55 and 0.66 ± 0.51 respectively; P = 0.003) (Table 3).

Discussion

In this study, we addressed the common finding of low caries incidence in DS children compared to healthy children from the aspects of saliva biochemistry and mucosal pH.

Both the study and the control groups were subdivided in relation to their caries status, in order to investigate the influence of each parameter in the study groups on the systemic condition and the caries status.

Our findings are in accordance with some previous reports that found 56% of DS individuals to be caries free[2,4–9]. We also agree with Morinushi et al.[5] who reported higher incidence of caries free children under the age of five years. In the present study, we

Table 3. PI and GI in the examined groups.

(mean ± SD, [range])	Down syndrome group (Ds)		Control group (C)	
	Caries free (DS-C)	Caries (Ds-CF)	Caries free (C-C)	Caries (C-CF)
PI	1.46 ± 0.55 [1.0–3.0]	1.04 ± 0.65 [0–3.0]*	1.11 ± 0.43 [0.5–2.0]	1.17 ± 1.03 [0–3.0]
GI	1.29 ± 0.55 [0–3.0]	0.9 ± 0.76 [0–3.0]	0.66 ± 0.51 [0–2.0]*	0.64 ± 0.75 [0–2.0]

*Significant difference from DS-Ca, ^Significant difference from DS-CaF, •Significant difference from C-Ca, ˣSignificant difference from C-CaF. *$P < 0.05$; **$P < 0.01$ (one-way ANOVA). Values are indicated as mean ± SD (range).

clearly demonstrated that younger children whether healthy or with DS, were more likely to be caries free (Table 1). Obviously, younger age indicates shorter exposure of teeth to caries risk factors. In addition, DS children who have mental retardation are relatively more dependent on their caregivers in regard to nutrition and oral hygiene habits. It is logical to expect that in the younger children dietary and oral hygiene habits can be easier to control. Nevertheless, the differences between the caries and the caries-free children with DS were not so prominent. It seems that other mechanisms beside nutrition and oral hygiene may be involved in the caries process of this population.

Moreover, as reported by several authors[2–4] we also found higher scores of PI in DS-Ca group (Table 3). As stated before, this finding could be attributed to the difficulties of the caregivers to brush the children's teeth.

GI scores in our study were also in accordance with previous reports that found more periodontal treatment needs among patients with DS[2].

The ionic composition of saliva changes normally: while primary saliva resembles the plasma or the interstitial fluid, with maturation, the fluid is modified by an active transport of solutes in the duct system of the gland tissue[17].

We found higher levels of Cl in the DS groups, especially among the DS-Ca children. Cl concentration in saliva is normally lower than in the serum, due to active absorption of Cl. Moreover, the concentration of Cl is higher in stimulated saliva[17]. Jara *et al.*[29] could not find any differences in Cl concentration between DS and normal individuals; however, they limited their measurements only to stimulated saliva collected from the parotid glands. In our study, unstimulated whole saliva was analysed, where differences in Cl concentrations were prominent. Winer *et al.*[30] also found lower Cl concentration in both submandibular and parotid saliva.

Therefore, our findings are not in agreement with Jara *et al.*[29] and Winer *et al.*[30] and show a dramatic increase in Cl concentration in the DS children. It is possible that a disruption in the mechanism of Cl reabsorption that may be part of the trisomy is expressed in this population, that modifies the acinar transfer ion mechanism, hence, alters the salivary anion levels.

Salivary Ca concentration in DS population was examined by several authors and most of them did not find any difference between DS and healthy populations[13,19,19,30]. We found significantly higher levels of Ca in the DS groups compared to healthy children and also significant differences in the subgroups. Routinely, Ca is actively secreted from acinar cells, and is influenced by the concentrations of NaCl and water that are absorbed by the duct system. We assume that higher levels of Ca may be attributed to lower levels of Cl secretion, in a compensatory mechanism to preserve saliva osmolarity, or a similar reflection, as seen with Cl to modifications of ion transfer mechanism.

In our study, Na and K could be measured only in the DS population. Interestingly, the same trend was observed in these cations, i.e. their concentrations were significantly higher in the DS-Ca group compared to DS-CaF group. The literature demonstrates lack of agreement regarding the concentrations of Na and K in DS population. While Seiqura *et al.*[13,21] showed higher concentrations of salivary Na and lower K concentrations compared to healthy subjects, Jara *et al.*[29] and Winer *et al.*[30] found the contrary. We examined the concentrations of these cations in relation to caries status in DS children only. K can be used as an indicator to acinar cell electrolyte metabolism, since it is secreted actively by acinar cells and its concentration in saliva is higher than in the plasma. However, Na secretion mechanism is totally different; the cation is secreted passively and is totally dependent on the concentrations of other ions. Salivary Na concentration is usually lower than in the plasma.

We found that the concentrations of Na and K were elevated in the DS-Ca group. We hypothesize that these cations act in response to increased Cl (their counter anion) concentration. We cannot comprehend exactly how and where the acinar cell metabolism is disrupted, but alterations in the salivary ion concentration in the DS

group seem evident. We believe that the trisomy in DS manifests itself in the salivary glands, and as a result, a different salivary environment of electrolytes is created, that interferes in the caries process.

Another important factor in the caries pathogenesis is the pH. In our study we measured pH values in four sites of the oral mucosa. These sites map the oral cavity and support the concept that the oral cavity is a collection of distinct micro-environmental compartments[22].

Most studies that examined salivary pH and buffering capacity have traditionally measured it from saliva collected intra-orally and analysed extra-orally, a fact that may have led to inaccurate results for the following reasons[1]: pH values are actually an average of whole saliva and do not represent the different intra-oral micro environment[2], the buffering system may alter once saliva is taken out of the oral cavity, and [3] the salivary film, covering the soft and the hard tissues may not precisely correspond to the secreted saliva in regard to pH levels[4,7,14].

Four intraoral sites in the hard palate: the right and left buccal mucosa and the middle tongue, were selected since the oral cavity is composed of several micro niches characterized by different features among which is pH values as previously described by Aframian et al.[22] Consequently, the right and left buccal sites are adjacent to the orifices of the Stensen's ducts therefore representing the pH of saliva secreted by the parotid glands. On the other hand, the sites on the palate and on the tongue represent minor salivary glands' secretion. Therefore the mean of these four sites represent a good picture of the mucosal intra-oral pH levels.

Our results demonstrated significant lower pH values in all examined sites in DS population in general and specifically in DS-Ca individuals thus, are in agreement with previous studies[2,7,13,21]. Furthermore, DS-Ca patients presented the lowest pH values, followed by the DS-CF group which exhibited higher values, then by the C-Ca group. The highest pH values were found in all sites in C-CaF group.

It appears that DS subjects have a more acidic oral environment than healthy subjects; yet they demonstrate lower caries incidence. Not only we could not show a better salivary buffering capacity as published by Siqueira et al.[6] and Yarat et al. [14] the lower pH values which were found in the DS-CaF group emphasize that this was probably not the etiologic factor for the low caries incidence. We think that the conflicting reports in the literature about the buffering capacity in DS may be attributed to different methods of measurements.

The present study faces a limitation: The mean age of the control group was higher than the mean age of the studied group. This was due to the fact that cooperation of very young children who attend a clinic for a routine dental examination is very limited, in particular for pH examination and saliva collection.

Our findings emphasize the difference in the oral health environment within the DS population and may open new strategy to maintain the oral health of this affected population. Yet, further investigation of the complex interrelations of the various factors that may be involved in the different caries levels of DS and healthy individuals is needed.

Conclusions

Both pH and sialochemistry (Cl, Ca, Na and K levels) were different among children with DS with caries compared to healthy children.

The differences between DS and healthy children were more prominent in relation to caries status.

All variables, taken together, could not exactly explain the overall low caries rate of children with DS, thus only demonstrates the complex nature of dental caries. We believe that the trisomy in DS manifests itself in the salivary glands. As a result, a different salivary environment of electrolytes is created, that interferes in the caries process, leading to lower caries rates among DS children.

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