Caries prevalence, level of mutans streptococci, salivary flow rate, and buffering capacity in subjects with Down syndrome

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Abstract
The aim of this study was to correlate caries experience and physiological and microbiological profiles. The study group comprised 60 individuals with Down syndrome, both genders, aged from one to 48 years. The prevalence of caries was analyzed by DMFT/DMFS and dmft/dmfs indexes. Physiological factors such as flow rate, and buffer capacity and microbiological factor such as mutans streptococci counts were observed. The average DMFT and DMFS were, respectively, 4.53 and 6.85, whereas the mean dmft and dmfs values were 1.55 and 2.55, respectively. Ninety-four percent of 18 individuals that saliva was possible to collected presented low flow rate and only 6% of them had normal flow rate; 44% percent had low buffer capacity, 39% had limited buffer capacity and 16% had normal buffer capacity. Sixty percent of individuals had high values of CFU/mL (>1.000.000 *S. mutans*); while 40% presented low values of microorganisms (<100.000 *S. mutans*). Data of clinical, physiological and microbiological characterization were statistically analyzed through Pearson's correlation and Chi-square test. A p-value d" 0.05 was considered significant. DMFT/DMTS and dmft/dmfs indexes increased with age. Pearson's correlation showed significant values to DMFT/DMFS x age (r= 0.80 and r= 0.82; p< 0.01). Flow rate and buffering capacity were low. Individuals had high mutans streptococci counts (CFU/mL). DMFT/DMFS did not present significant correlation with flow rate, buffering capacity and mutans streptococci counts and no association with gender. The prevalence of dental caries increased with age at individuals with Down syndrome. As caries is a multifactor disease, other factors, which were not evaluated in the present study, such as diet, host and oral hygiene might be influencing the development of dental caries in these individuals.

Key Words:
caries, flow rate, buffer capacity, *mutans* streptococci, Down syndrome

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Introduction

The world literature reports the low caries prevalence and high periodontal disease prevalence in Down syndrome population when compared to individuals with mental delay, or normal persons[1-8].

Many studies state that low caries prevalence may be related to eruption delay and saliva compounds of these children[9-11], to dental morphology with less pronounced pits and fissures and less accidental occlusal surface by bruxism[1,12-13] or by different microbiota associated to dental biofilm[13].

On the other hand, some authors do not observed statistical difference when the caries disease and index were correlated in both control and Down syndrome group[14-15].

Flow rate and buffer capacity of saliva have an important role at organization of oral microbiota because they keep the saliva pH constant when low acid is added. These can avoid the colonization of some pathogenical bacterial in mouth and can remove and buffer some acids that are produced by these microorganisms[16].

Mutans streptococci counts are important to verify the development of caries risks because they are early colonizers and add glycoprotein in salivary pellicle over the dental enamel by an enzymatic system to change saccharose and the production of glucosyltransferases and synthesize glicans of high molecular weight[16].

Down syndrome individuals can have physiological alterations of flow rate and saliva composition, which are fundamental to microorganisms' colonization, actuating directly at the beginning of pathological process like caries disease.

Thus, the aim of this study was to examine salivary flow rate and buffer capacity, to determine microbiological profile by mutans streptococci counts and correlate these dates with the prevalence of caries disease in Down syndrome.

Material and Methods

Study group

This study was submitted and approved by Research Ethic Committee of the Sagrado Coração University, Bauru SP, Brazil. All volunteers and their parents/guardians were informed about the aim of the study and authorized the clinical examination and saliva sample collection.

The study group comprised sixty individuals with Down syndrome of both genders, aged from one to 48 years old. They were patients of the Program of Assistance for Patients with Special Needs at Sagrado Coração University (PAIPE – USC) and students from a special school in Bauru – São Paulo (APAE).

This sample corresponds to about 90% of individuals with Down syndrome of PAIPE and 49% of APAE - Bauru students. Individuals who had taken antibiotics during the month prior to the examination, with several mental delay or orthodontic treatment were excluded.

The volunteers were divided in six groups, according to the age to compare the data: from one to five years old; from six to ten years old; from 11 to 15 years old; from 16 to 20 years old; form 21 to 27 years old and from 31 to 48 years old.

Since the aim of this study was to identify the physiological and microbiological factors in Down syndrome in order to verify its association with dental caries, control group was not included. Furthermore, literature demonstrated that many similar studies were performed in children without Down syndrome which points that another comparative study was not necessary.

Clinical examination

The examiner was trained and calibrated according to World Health Organization (WHO) one month before clinical examination. At this occasion, at least 13% of the sample was examined twice, in consecutives days to prove the calibration of intra-examiner by Kappa test, which is considered as great concordance when the interval was 0.81 – 0.99. The obtained Kappa value was 0.92.

Clinical examination was performed in 59 individuals and the DMFT/DMFS and dmft/dmfs indexes were determined according to WHO. Subjects were clinically examined on a dental chair with artificial light using a sickle-shaped explorer and a dental mouth mirror after dry the tooth with air. No information about diet and hygiene was providing before examination to volunteers.

DMF-T index analyzes the caries at permanent dentition. The initials represent respectively: caries teeth (D), missed teeth (M), filled teeth (F) and the unit is the teeth (T). The dmf-t index corresponds to DMF-T in relation to temporary dentition including only caries teeth (d), indicated extraction (m) and filled (f). It excludes the extracted ones, regarding the difficulties to identify lost teeth due to caries or the natural process of dental exfoliation.

The rates from DMF-T and dmf-t were obtained by division of all the teeth attacked for the number of examined individuals. The DMF-S and dmf-s indexes are refined alternatives to evaluate the caries emergence and their unit measure is the surface of the teeth(s). The criteria to evaluate were the same to DMF-T and dmf-t.

Saliva collection

Saliva samples were collected between 8 am and 9 am or between 1:30 pm e 2:30 pm, according to the appointment.

Salivary flow rate determination

A piece of paraffin gum-base of approximately 1.5 g. was supplied to each volunteer. Each individual chewed the gum for about 30 seconds. Initial stimulated saliva was swallowed and, after that, the volunteers chewed the gum for from 3 to 5 minutes and saliva samples of approximately 1.5 ml were collected in sterile glass tubes with thread, to salivary and

microbiological analysis. The expelled air was introduced inside the tubes. The tubes were closed and carried to the Molecular Biology Laboratory at University of Sagrado Coração. Samples collected were kept in ice. The time of samples collect until the processing did not exceed 2 hours[17]. The average flow rate was calculated from the total volume by time of secretion (<0.1 mL/min – xerostomia; 0.1“% 0.7 - pronounced low; 0.7“% 1.0 – low flow rate; 1.0“%$%2.0 normal flow rate; >2.0 mL/min – high flow rate)[18].

Only 18 individuals participated in stimulated saliva collection because the deficiencies caused by Down syndrome, such as absence of chewing swallowing, expectoration reflexes and children age.

No stimulate saliva was collected with sterile swab at individuals (n=40) who could not chew gum or expectorate the saliva. The swab was weighed before and after collection and the difference was the same for all colleted saliva. The saliva collected with Swab was diffused at 1mL sterile distillated water to obtain 1 mL of saliva to perform the dilutions to microbiological analysis.

Buffering capacity and mutans streptococci *counts*

Saliva samples were analyzed for pH and buffer capacity by using pH paper (pH-Fix 0-14, MN, Duren, Germany, Germany)[14]. Buffer capacity was determined by 0.5 mL of collected saliva in sterile glass tubes with 1.5 mL HCL 5 mM. The tubes were shaken for a minute, and opened to remove CO_2. The pH final was determined after 5 minutes by pH paper[14] (pH from 5 to 7 - normal buffering capacity; pH from 4 to 5 – limited values; pH < 4 - low buffering capacity)[18]. Thus, saliva samples were diluted in a decimal series (from 10^{-1} to 10^{-4}) in 0.05 M phosphate buffer, pH 7.3.

Aliquots of 25 mL of each dilution (n=58 patients) of saliva were inoculated in Bacitracin 0.2UI/mL Mitis Salivarius - MSB agar with sucrose 5%[16] to the culture of mutans streptococci. The plates were incubated for 48h in candle jars at 37ºC. After this period, the colonies of *mutans* streptococci were counted to determine the CFU/mL number (> 1.000.000 *S. mutans* - high CFU/mL; <100.000 *S. mutans* - low CFU/mL) (Table 3). The observation and colonies count characteristics of mutans streptococci group (white-grayish colonies with granular surface similar grind glass, with or without extra cell polysaccharide drop on the top) were performed under reflected light with a stereoscopic microscope (Zeiss, Thornwood, New York, United States of America), according to standard described to MSB agar[19-22].

Statistical analysis

Clinical (DMFT, DMTS, dmft, dmfs), physiological (flow rate and buffer capacity) and microbiological characterization (*mutans* streptococci counts) were submitted to descriptive statistical analysis. For comparisons of the distribution between genders the Chi-square test was applied, and Pearson’s correlation was used for correlation among variables (p-value < 0.05).

Results

According to the results of caries indexes, study group had average values by 4.53 and 6.85 for DMFT and DMFS, respectively, whereas dmft and dmfs were 1.55 and 2.55, respectively. Twenty-one (36%) individuals were caries free; 17 (29%) of them were children (from one to 12 years old) and 4 (7%) were adults.

The average values of DMFT, DMTS, dmft, dmfs indexes, according to age are indicated at Table 1.

Table 1 – Average of DMFT, DMFS, dmft, dmfs, according to the age

	DMFT	DMFS	dmft	dmfs	n
1 - 5	-	-	0.44	0.44	09
6 - 10	0.65	0.65	1.85	3.05	20
11 - 15	1.60	1.80	2.14	3.60	10
16 - 20	6.00	8.14	0.00	0.00	07
21 - 27	9.00	11.43	3.00	7.00	07
31 - 48	13.50	26.00	-	-	06

Salivary analysis was performed in 18 individuals (30%) due to the difficulty to collect saliva in the other ones, such as the absence of chewing, swallowing saliva and expectoration reflexes. Four of them were children aged from eight to 12 years and 14 were adults aged from 14 to 48 years. Seventeen individuals (94%) had low flow rate (0.1-0.7 mL/min) and only one (6%) had normal flow rate (1.0 - 2.0 mL/min). Buffer capacity was low (pH < 4) for eight subjects (44%), limited (pH 4- 5) for seven (39%) and only three (7%) showed normal values (pH 5 - 7) (Table 2).

Mutans streptococci colonies were determined in 58 (96%) individuals. Eighteen (30%) had stimulated saliva collected and 40 (70%) had no stimulated saliva collected with Swab. Two samples were excluded from the study by contamination after laboratorial process. From these 58 individuals, 35 (60%) showed high values of CFU/mL (> 1.000.000 *S. mutans*); while 23 (40%) had low values of microorganisms (<100.000 *S. mutans*) (Table 3).

There was a positive significant correlation between DMFT and age (r= 0.80; p<0.01). Nevertheless, there was not correlation between DMFT and buffer capacity, flow rate and number of mutans streptococci colonies – CFU/mL (p>0.05).

There was a positive significant correlation between DMFS and age (r= 0.82; p<0.01) by Pearson’s correlation. DMFS index did not show correlation with buffer capacity, flow rate, and CFU/mL (p>0.05).

The relation of dental caries with salivary and microbiological

Table 2 – Analysis of salivary parameters of stimulated saliva (n=18)

Age	mL/min	n (%)	pH	n (%)
1-5	-	-	-	-
6-10	0.1 + 0.7	02 (11.0)	5 - 7	01 (5.6)
			4 - 5	02 (11.0)
11- 15	0.1 + 0.7	03 (16.7)	4 - 5	03 (16.7)
	1.0 +¦ 2.0	01 (5.6)	< 4	01 (5.6)
16- 20	0.1 + 0.7	04 (22.2)	5 - 7	01 (5.6)
			4 - 5	01 (5.6)
			< 4	03 (16.7)
21- 27	0.1 + 0.7	03 (16.7)	< 4	03 (16.7)
31- 48	0.1 + 0.7	05 (27.8)	5 - 7	01 (5.6)
			4 – 5	02 (11.0)

Table 3 – Mutans streptococccci counts at saliva (CFU/mL)

CFU/mL	Stimulated Saliva	Non stimulated saliva
	n (%)	n (%)
>1.000.000 *S. mutans*	17 (94)	18 (45)
<100.000 *S. mutans*	01 (6)	22 (55)

parameters is demonstrated at Table 4.

When the distribution of DMFT and DMFS index were associated with genders by Qui-square test, there were not significant statistically difference (p>0.05).

Discussion

In this study, caries indexes of individuals with Down syndrome were high. Other study verified that the percentage of children with Down syndrome caries free were higher than all ages analyzed (5, 8, 12, 15 years old) when compared to children with other special needs[23]. High values in our study are possibly due to the largest sample that analyzed children and adults too. In addition, it may be possible that children analyzed in that study did not present completed dentition at the moment of clinical examination because the eruption delay at Down syndrome[9-11]. On the other hand, prevalence of caries by DMFS was not verified, which is a pure alternative to evaluate the caries' incidence. The values observed may not be the same of reality once the authors verified the occurrence of caries by teeth not by teeth surface, justifying the high values that we found.

Many other studies found DMFT= 2.68; 3.96 and 0.10, respectively, for Down syndrome individuals[6,8,15]. The population at the study of Moraes et al.[15] did not get dental treatment. Maybe this fact influenced at values to filled and missed teeth. In the study of Vázquez et al.[6], individuals had benzodiazepinics that may have affected caries indexes. Individuals that had drugs previously the clinical examination

Table 4 - Analysis of salivary and microbiological parameters, and age and gender in relation to caries indexes

Parameters	DMFT	DMFS	dmft	dmfs
Flow rate*	p>0.05	p>0.05	p>0.05	p>0.05
Buffering Capacity*	p>0.05	p>0.05	p>0.05	p>0.05
CFU/mL*	p>0.05	p>0.05	p>0.05	p>0.05
Age*	r= 0.80; p< 0.01	r= 0,82; p< 0.01	p>0.05	p>0.05
Gender†	p>0.05	p>0.05	p>0.05	p>0.05

** Pearson's correlation (p<0.05); † Chi-square test (p<0.05)*

and saliva collection were excluded to avoid wrong results at this study. Fung and Alllison[8], observed only delay and filled teeth so the aim of this study was different because it included missed teeth. The analysis in this study was more detailed and the highest number of parameters justifies the highest values to the caries indexes. Although the caries indexes of those studies were low when compared to the results of Table 1, those authors did not use DMFS, so it embarrass the comparison of the results and indicates the cause of divergences at values.

The kind of dentition may affect DMFT probably because the high time of teeth exposure at mouth[24]. Results showed that many volunteers were adults. Thus, caries indexes were so high because the age and the time of exposure to local factors predisposed to development of caries lesions. Furthermore, the age supports the occurrence of periodontal disease and it promotes the absence of teeth. DMFT and DMFS indexes were positively correlated to age.

When salivary parameters were verified in this study almost all individuals had low flow rate, as found in other studies[25-28]. Although low flow rate suggests caries risk increasing, since saliva has a fundamental role to clean the mouth and to remove food and bacterial leftovers[29], it did not influence at high caries indexes observed in this study. DMFT and DMFS indexes were not correlated to flow rate.

Most of patients (44%) in this study had low buffer capacity. Other studies showed higher buffer capacity in Down syndrome when it was compared to individuals without the syndrome[25,28]. Although the buffering capacity was low, it does not seem to influence caries prevalence once these factors were not correlated either.

Dental caries is closely associated with *mutans* streptococci and *Streptococcus mutans* and *Streptococcus sobrinus* have been frequently found at the mouth of individuals with high activity of caries[29]. Many studies report the correlation between high dental caries prevalence and the presence of *S. mutans*[30-33]. Although more than a half of volunteers (60%) of this study had high number of CFU/mL, this factor seems to have no influence on caries indexes due to the correlation has not been found between counts of total colonies of mutans streptococci and the caries indexes observed. The negative correlation found between caries indexes and counts of total colonies of mutans streptococci may confirm the multifactorial caries etiology when the present sugar at host's diet associated to high counts of these microorganisms are fundamental to development of the pathology[34].

Gender has not affected the caries prevalence at this population.

This study showed that prevalence of dental caries increased with age in individuals with Down syndrome. Flow rate and buffer capacity were low. In addition, the number of mutans streptococci was high at mouth. As caries is a multifactorial disease, other factors, which were not evaluated in the present study, such as diet, host and oral hygiene might be influencing the development of dental caries in these individuals.

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