Revista Odontológica Mexicana

Facultad de Odontología

Vol. 20, No. 3 • July-September 2016

pp e155-e161

ORIGINAL RESEARCH

Salivary profile and its relation to CEFT index in 5 year old children

Perfil salival y su relación con el índice CEOD en niños de 5 años

Antonio Armando Aguirre Aguilar,* Fraysy Graciela Narro Sebastian§

ABSTRACT

Objective: To determine relationship of salivary profile with CEFT (carious, extracted, filled, teeth) index in five year old children. **Material and methods:** A cross-sectioned study with probability sampling was conducted in 40 children. Children were divided into fourth groups of ten subjects each, according to CEFT index. Sample harvesting was conducted according to the non-stimulated saliva method. The following measurements were conducted: salivary volume, salivary flow, *Streptococcus mutans* population density, salivary pH, salivary buffer ability and fluoride level. **Results:** Average salivary profile was established according to the following values: 4.76 mL salivary volume, 0.48 mL/min salivary flow, 4.85×10^5 CFU/mL S*treptococcus mutans* population density, 6.75 salivary pH, 5.9 salivary buffer capacity and 0.04997 fluoride level. Values per salivary parameter at all caries levels did no significantly differ: $p > 0.05$. **Conclusions:** In five year old children, salivary profile did not significantly change at different levels of dental caries.

RESUMEN

Objetivo: Determinar la relación del perfil salival con el índice CEOD en niños de cinco años. **Material y métodos:** Se realizó un estudio con muestreo probabilístico y de corte transversal en 40 niños, divididos en cuatro grupos de 10 individuos cada uno, de acuerdo al índice CEOD; la recolección de muestras se realizó mediante el método de saliva no estimulada, procediendo a la medición del volumen salival, flujo salival, densidad poblacional de *Streptococcus mutans*, pH salival, capacidad buffer salival y nivel de flúor. **Resultados:** Se estableció un perfil salival promedio con los siguientes valores: volumen salival de 4.76 mL, flujo salival de 0.48 mL/min, densidad poblacional de *Streptococcus mutans* de 4.85×10^5 UFC/mL, pH salival de 6.75, capacidad buffer salival de 5.9 y nivel de flúor de 0. 04997 ppm; y los valores por parámetro salival en todos los niveles de caries no presentaron diferencia significativa: $p > 0.05$. **Conclusiones:** El perfil salival no difiere de manera significativa en los diferentes niveles de caries dental en niños de cinco años.

Key words: Saliva, dental caries.
Palabras clave: Saliva, caries dental.

INTRODUCTION

The great frequency of oral and dental infectious diseases has been the main driving force in the instauration of preventive methods in Health Providing Facilities. New study methods such as research conducted on saliva functions and their importance in dental health have been explored. Nevertheless, there are few studies relating salivary profile and caries prevalence in children, this might be of the utmost importance in a future establishment of progress concerning dental caries.[1-3]

Presently, saliva use represents a promising alternative to diagnose and monitor the evolution of certain diseases, since variations in saliva's chemical composition and common components might considerably alter health. Moreover, accessibility, positive correlation of multiple parameters with respect to serum and absence of invasive methods used for its harvesting are all advantages offered by saliva as a diagnostic means.[4-7]

Salivary profile is composed by the mean values of its main characteristics; these characteristics are pH, volume, salivary flow, buffer ability, salivary population density of *Streptococcus mutans* and fluoride values.[8]

In general terms, daily adult saliva production is 1 -1.5 liters; in a five year old child an average flow of 0.62 mL/min, equivalent to 0.89 liters a day has been reported.[8] This salivary flow is subjected to a series of changes caused by age, gender, body weight, number of teeth present in the mouth, food intake, circadian

* DDS, Professor at the Stomatology, Graduate School and Stomatology Specialization Second Unit at the National University of Trujillo.
§ DDS graduated at the Stomatology School of the National University of Trujillo.

Received: March 2015. *Accepted: August 2015.*

This article can be read in its full version in the following page:
http://**www.medigraphic.com/facultadodontologiaunam**

See related content at doi:
http://dx.doi.org/10.1016/j.rodmex.2016.08.011

rhythm as well as oral diseases. Likewise, increased salivary secretion takes place during tooth eruption periods due to the hyper-stimulation of oral mucosa's peripheral receptors.[4,7,8]

It can be said that pH is a measuring unit studied in many research projects since it expresses the degree of acidity or alkalinity of a substance. It is composed of a scale of values, graded from 0 to 14, neutral being an average of 7.0. In healthy circumstances, salivary pH at rest is kept at a narrow range of 6.7 to 7.4; in children this pH has a 6.94 average value.[8-11]

In cases when oral hygiene is not suitably observed, pH within the mouth becomes acid and enables development of several oral diseases. Nevertheless, saliva exerts comprehensive protection of the dental enamel, by stabilizing Ph in the mouth. This is known as salivary buffer capacity. It has a 5.9 pH average value in caries-free children.[8,10]

Streptococcus mutans is the most important and studied bacterium found in the mouth, since it is able to ferment different sugars and generate lactic acid (acidogenic capacity). *Streptococcus mutans* population density is described as the amount of salivary bacterial factors able to produce oral diseases. In caries-free children, this population density is established at an average 12×10^4 UFC/mL.[8,9,12]

Fluoride present in saliva enhances re-mineralization, inhibits demineralization and bacterial enzymes. Its concentration in ductal saliva varies from 0.006 to 0.016 parts per million (PPM) according to whether it is found in areas with or without drinking water fluoridation. In caries-free children, it has been established that fluoride level in non stimulated saliva reaches an average value of 0.058 ppm.[8,10,13,14]

Since salivary profile in caries-free children has already been determined,[8] the present research project targeted determination of profile of five year old children with caries, according to affectation levels employed in the CEFT index.

MATERIAL AND METHODS

The present prolective, cross-sectioned, comparative and observational research was conducted at the Health Facility number 252, «Niño Jesus» of the Trujillo district. Saliva samples of 40 children were used. The sample was determined through the formula that compares two or more study groups when quantitative variable measure is evaluated.

Inclusion criteria were the following: five year old boys and girls with very low, low, moderate, high and very high levels of CEFT, full primary dentition, apparent general good health, nose breathers, not consuming drugs that might interfere with salivary functions. Children's parents had to grant consent for their participation in the study.

Sample harvesting

Four groups of ten children each were formed according to their CEFT index (very low/low, moderate, high, very high). This was determined through oral inspection and caries prevalence record according to CEFT index. Severity was determined by values obtained in the CEFT index: Very low and low risk (≤ 2), moderate risk ($> 2 \leq 4$), high risk ($> 4 \leq 6$, very high risk (> 6).[15]

The fact that the children had not eaten in the two hours prior to the sample harvesting was taken into consideration; children were asked to rinse their mouth with fresh water so as to eliminate any food residue. Children were seated in a 90° position, then saliva samples were taken during a two minute period (without swallowing); this was achieved with a gauged saliva collecting glass, such as is indicated in the Tomas Seif[16] established protocol for collection of non-stimulated saliva. Samples were then labeled and taken to the laboratory where they were suitably stored to later measure selected salivary parameters.

Recording, in millimeters, was achieved in gauged collecting glass. Record of salivary flow was obtained by dividing by two the amount of saliva obtained in the gauged container (non stimulated salivary flow per minute).

Registration of *Streptococcus mutans* population density was conducted with a laboratory test called «isolation and quantification of *Streptococcus mutans* present in saliva». This technique consisted on performing dilutions of 1:10, 1:100. 1:1,000 until reaching the suitable number of solutions which might guarantee precise and clear count. This was achieved by placing 100 μL of salivary sample in 900 μL of sterile isotonic saline solution. Of the last three dilutions 100 μL were seeded in Petri dishes with an agar rake containing TYS20B, a selective medium for the development of *S. mutans* colonies. The sample was incubated in an oven at 37 °C during 48 hours in anaerobiosis circumstances. Colony quantification was achieved with the surface method; they were evaluated in UFC/mL.

HANNA H198128 potentiometer was used to record salivary pH. It was gauged every 10 samples. Following manufacturer's instructions, salivary pH of all samples was recorded with a manufacturer-prepared substance. All registries were entered in their respective record sheets.

Buffer capacity recording was achieved by the Ericsson method. This method consisted on transferring 1 mL saliva to 3 mL HCl (0.0033 mol/L) for non-stimulated saliva, with a 20minutes mixing time to eliminate CO_2. After this, salivary pH in saliva was measured using a pH-meter.

Finally, to record fluoride levels, a potentiometric measurement was conducted. From it, information of fluoride ion concentration in saliva was obtained. Measurements were taken as follows: 5 mL of saliva were measured and 5 mL of TISAB II within a tres ounces polyethylene glass. After this the electrode was placed into the prepared solution which was then shaken until readings were stabilized, at that moment values were accepted.

Data, recorded in data collection cards, were tabulated following an authorized tabulation pattern to assists statistical package SPSS-20.0. Statistical analysis included results of mean and standard deviation for each variable of the salivary profile. For comparison purposes, test F of variance analysis was used, since it was considered that there was significant difference when error probability is lower than 5% ($p < 0.05$).

RESULTS

The present study assessed salivary volume, salivary flow, *Streptococcus mutans* population density, salivary pH, salivary buffer capacity and fluoride level in the saliva of 40 5-year old children with caries. Subjects were divided into four groups in order to establish a relationship with CEFT index.

According to CEFT index, mean non-stimulated salivary volume in milliliters was the following: very low/low group: 4.39 ± 1.50; moderate group: 5.60 ± 1.68; high group: 5.56 ± 4.36; very high group: 3.49 ± 2.00; $F = 1.473$ and $p < 0.05$ *(Table I)*.

It was equally determined that non-stimulated average salivary flow (in mL/min) was the following according to CEFT index: very low/low group: 0.44 ± 0.15, moderate group: 0.56 ± 0.17, high group:0.56 ± 0.44 and very high group 0.35 ± 0.20; $F = 1.463$ and $p < 0.05$ *(Table II)*.

According to CEFT index, average *Streptococcus mutans* salivary population in non-stimulated saliva was the following: very low/low group 3.40×10^5 CFU/mL ± 209000; moderate group: 4.25×10^5 CFU/mL ± 270000, high group: 4.97×10^5 CFU/mL ± 323000, and in very high group 6.76 × 10% CFU ± 648000, $F = 1.274$ and $p < 0.05$ *(Table III)*.

According to CEFT index average in non stimulated saliva was the following: very low group: pH of 6.83 ± 0.54, moderate group: pH of 6.83 ± 0.54, high group: pH of 6.85 ± 0.35, very high group. pH of 6.55 ± 0.53; $F = 0.608$ and $p < 0.05$ *(Table IV)*.

With respect to non-stimulated saliva buffer capacity according to CEFT index, the following values were obtained: very low/low group 5.62 ± 0.46, moderate group: 5.55 ± 0.43, high group: 5.73 ± 0.34, and very high group 5.42 ± 0.46, $F = 0.882$ and $p < 0.05$ *(Table V)*.

Average fluoride level (in ppm) in non stimulated saliva according to CEFT index values were the following: very low/low group 0.0517 ± 0.0084, moderate group 0.0504 ± 0.0056, high group 0.0499

Table I. Non-stimulated average salivary volume according to CEFT index in five year old children.

Indicador	CEFT index			
	Very low/low	Moderate	High	Very high
	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Non stimulated salivary volume (mL)	4.39 ± 1.50	5.60 ± 1.68	5.56 ± 4.36	3.49 ± 2.00

$p < 0.05$, $n = 10$.

Table II. Non stimulated average salivary flow according to CEFT index in five year old children.

Indicador	CEFT Index			
	Very low/low	Moderate	High	Very high
	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Non stimulated salivary flow (mL/min)	0.44 ± 0.15	0.56 ± 0.17	0.56 ± 0.44	0.35 ± 0.20

$p < 0.05$, $n = 10$.

± 0.056 and very high group 0.0479 ± 0.0058, F 0.608 and p < 0.05 *(Table VI)*.

Comparison of all salivary parameters found at different caries levels established p < 0.05 for all *(Table VII)*.

Thus, a mean salivary profile in children with caries was established at a 4.76 salivary volume, a 0.48 mL/min salivary flow with a population density of *Streptococcus mutans* of 4.85 × 10^5 CFU/mL, 6.75 salivary pH, 5.58 buffer capacity and 0.04997 fluoride presence in saliva *(Table VIII)*.

DISCUSSION

The importance of saliva and its functions in oral health preservation is well known, even more so as a diagnostic tool for different oral conditions, nevertheless, research targeting the establishment of different relationships among characteristics of a salivary profile and caries in children are scarce.

In the present study, no significant difference was established among the values of the four groups which were classified according to CEFT index for non-stimulated salivary volume. Therefore, no significant difference was observed among values of non-stimulated salivary flow according to CEFT index. This total mean value was found to be above the generally assigned value in human beings which is from 0.25 to 0.35 mL/min in a resting (quiescent) state.[7] This is probably due to the general aspects of sample inclusion. Nevertheless, more abundant salivary flow was established in children under 8 years of age, when compared to adults; this might be due to the level of functional maturity progressively reached by salivary glands and muscles of the oral region.[17] In a study conducted in 2014 by Aguirre A

Table III. Mean population density of *Streptococcus mutans* in non-stimulated saliva according to CEFT index in five year old children.

Indicador	CEFT index			
	Very low/low	Moderate	High	Very high
	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Streptococcus mutans population density (CFU/mL)	340000 ± 209000	425000 ± 270000	497000 ± 323000	676000 ± 648000

p < 0.05, n = 10.

Table IV. Mean pH in non-stimulated saliva according to CEFT index in five year old children.

Indicador	CEFT Index			
	Very low/low	Moderate	High	Very high
	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Non-stimulated salivary pH (pH units)	6.77 ± 0.75	6.83 ± 0.54	6.85 ± 0.35	6.55 ± 0.53

p < 0.05, n = 10.

Table V. Mean buffer capacity in non stimulated saliva according to CEFT index in five year old children.

Indicador	CEFT index			
	Very low/low	Moderate	High	Very high
	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Buffer capacity of non stimulated saliva (pH units)	5.62 ± 0.46	5.55 ± 0.43	5.73 ± 0.34	5.42 ± 0.46

p < 0.05, n = 10.

Table VI. Mean fluoride level in non-stimulated saliva according to CEFT index in five year old children.

Indicador	CEFT index			
	Very low/low	Moderate	High	Very high
	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Fluoride level in non stimulated saliva (ppm)	0.0517 ± 0.0084	0.0504 ± 0.0056	0.0499 ± 0.0056	0.0479 ± 0.0058

$p < 0.05$, $n = 10$.

Table VII. Comparison of mean level salivary profiles according to CEFT index in five year old children.

Salivary profile	U_1	U_2	U_3	U_4	F test of variance analysis	Significance for Ho: $U_1 = U_2 = U_3 = U_4$	Total population mean
Salivary volume (mL)	4.39	5.60	5.56	3.49	F = 1.473	0.238 p > 0.05	4.76
Salivary flow (mL/min)	0.44	0.56	0.56	0.35	F = 1.463	0.241 p > 0.05	0.48
Salivary density of *S. mutans* (CFU/mL)	3.40×10^5	4.25×10^5	4.97×10^5	6.76×10^5	F = 1.274	0.298 p > 0.05	4.85×10^5
Salivary pH (pH)	6.77	6.83	6.85	6.55	F = 0.608	0.614 p > 0.05	6.75
Buffer capacity	5.62	5.55	5.73	5.42	F = 0.882	0.459 p > 0.05	5.58
Salivary fluoride (ppm)	0.0517	0.0504	0.0499	0.0479	F = 0.608	0.614 p > 0.05	0.04997

U_1 = Population mean value for very low/low CEFT.
U_2 = Population mean value for moderate CEFT.
U_3 = Population mean value for high CEFT.
U_4 = Population mean value for very high CEFT.

Table VIII. Salivary profile in five year old children.

Salivary profile	
Salivary volume	4.78 mL
Salivary flow	0.48 mL/min
Salivary density of *Streptococcus mutans*	4.85×10^5 CFU/mL
Salivary pH	6.75 pH
Buffer capacity	5.58 pH
Salivary fluoride	0.04997 ppm

and Rebaza L,[8] an average salivary flow at rest of 0.62 mL/min in caries-free five year old children was established. When comparing this result with results obtained in the present research, it can be observed that salivary flow in caries-free children is higher than average salivary flow of children with caries. This fact was equally supported by the study conducted by Marcano A et al (2009), who recorded a 0.36 mL/min salivary flow at rest in 5-11 year old children with dental caries, even though those children exhibited mixed dentition. Evidence in the sense that salivary flow is lesser in children with caries when compared to caries-free children is quite distinct, an explanation for this phenomenon could be provided by the direct relationship found between the amount of generated salivary flow and protective salivary factors produced when facing a critical decrease of salivary pH; likewise, in cases when saliva amounts are reduced there will be lesser lubricating function and longer retention time for food in the mouth[17] *(Tables I, II and VII)*.

Population density of *Streptococcus mutans* has been assessed due to the fact that this is the most important species associated to the initiation and development of dental caries.[9] Even though there is significant difference in population density according to

CEFT index, clear difference is observed in obtained values which increase according to increasing CEFT values.

Average population density recorded in children with caries is much higher than that obtained by Aguirre a and Rebaza L[8] (2014) in caries-free children, who reported a density of 12×10^4 CFU/mL. Even if we compare values of population density of the lower CEFT group with that obtained from caries-free children, the difference between their two values is still pronounced. This fact can be explained by the creation of favorable circumstances for *Streptococcus mutans* pathogenic multiplication and the later establishment and development of dental caries due to the metabolic acids[12] *(Tables III and VII)*.

Recording of mean salivary pH in children with caries shows a slightly lesser value with respect to the 6.94 pH recorded by Aguirre A and Rebaza L[8] (2014) in caries-free children, Nevertheless, they are found within the non-critical range of salivary pH at rest.[11,16] This pH difference might be due to the fact that in caries circumstances, acidogenic factors take precedence , and produce constant de-mineralization of the tooth´s inorganic matter, favoring thus the establishment of dental caries.[10] No statistically significant difference was observed among pH values according to CEFT index; the average value of the group with lower CEFT was almost similar to the average value of the group with higher CEFT *(Tables IV and VII)*.

It can be likewise observed that no statistically significant difference among buffer capacity values according to CEFT was found. Moreover, average value of buffer capacity in children with caries was lesser than average value in caries-free children.[8] This could be due to weaker salivary protective activity to counteract acids produced in the mouth, when compared to caries-free children, which would generate a constant demineralization process and would establish caries processes.[9,16] Nevertheless, according to Ericsson's classification and method, the mean value of buffer capacity in children found in the present study would define the existence of a «high» buffer capacity; this could probably be due to the fact that saliva would be exerting suitable protective action. Nevertheless, interaction of other factors such as micro-flora, diet and food impaction would possibly overcome buffer capacity and would initiate caries process[18] *(Tables V and VII)*.

Salivary fluoride level is important in the process of re-mineralization and dental caries prevention.[13] Mean salivary fluoride level obtained in the present study was 0.04997 ppm, its values decreased as CEFT increased. Nevertheless, no statistically significant difference was established among salivary fluoride values according to CEFT index. In this respect, Aguirre A and Rebaza L[8] reported a higher mean value in caries-free children.

It is important to note evidence of fluoride levels found in children with caries, taking into account the non-existence of a water fluoridation program in the water supply of the district of Trujillo *(Tables VI and VII)*.

Mean salivary profiles in children with caries present different values when compared to salivary profiles of caries-free children.[8] Difference among these values could be due to the establishment of the pathological condition of dental caries, which, due to its multi-factorial nature would elicit a physiological unbalance among salivary components and factors, generating and preserving certain oral circumstances which would favor its development[18] *(Table VIII)*.

CONCLUSIONS

Mean salivary profile in children with caries was the following: 4.76 mL salivary volume; 0.48 mL/min salivary flow, 4.85×10^5 CFU/mL *Streptococcus mutans* population density¸6.75 salivary pH, 5.58 buffer capacity and 0.04997 ppm fluoride level. No relationship between salivary profile and CEFT profile was found.

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Mailing address:
Antonio Armando Aguirre Aguilar
E-mail: clinicaaguirre@hotmail.com