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ORIGINAL RESEARCH

Dental caries and caries-associated mechanisms found in the saliva of first year students at the School of Dentistry, UNAM (National Autonomous University of Mexico)

Caries dental y microorganismos asociados a la caries en la saliva de los alumnos del primer año de la Facultad de Odontología, UNAM

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ABSTRACT

Three hundred and eighty first year students of the National School of Dentistry (UNAM) (n = 380) (academic year 2012-2013), were assessed targeting determination of DMFT (decayed, missing, lost teeth) index as well as to establish a relationship of whether caries is associated to *Lactobacillus* and *Streptococcus* microorganisms. DMFT index was recorded using World Health Organization (WHO) parameters. Samples of all students were taken and colony-forming units of *Streptococcus* and *Lactobacillus* were determined. DMFT indexes mean was established at 7.25 ± 4.59. Females (n = 278) and males (n = 102) exhibited mean DMFT indexes of 7.11 ± 4.66 and 7.29 ± 4.57 respectively. Results revealed that 19 year old students exhibited lesser amounts of caries than students of other ages. Both *Streptococcus* and *Lactobacillus* were significantly correlated to each other as well as to caries incidence. Increase in the number of the aforementioned micro-organisms, especially *Streptococcus mutans*, were associated to DMFT increase.

RESUMEN

Se estudiaron 380 alumnos del primer año en la Facultad de Odontología (n = 380) (periodo 2012-2013) a fin de determinar el índice CPOD y relacionar si la caries está asociada con los microorganismos *Streptococcus* y *Lactobacillus*. El índice CPOD (cariado, perdido y obturado) se registró usando los parámetros de la Organización Mundial de la Salud. Se tomaron muestras de saliva de cada alumno y se determinaron las unidades formadoras de colonias de *Streptococcus* y *Lactobacillus*. La media de los índices CPOD fue de 7.25 ± 4.59. Las mujeres (n = 278) y hombres (n = 102) presentaron una media de índices CPOD de 7.11 ± 4.66 y 7.29 ± 4.57, respectivamente. Encontramos que los alumnos de 19 años presentaron menos caries que los estudiantes de otras edades. Tanto *Streptococcus* y *Lactobacillus* se correlacionaron significativamente entre sí, así como en la incidencia de caries. Un incremento en el número de estos microorganismos, especialmente de *Streptococcus mutans*, se asociaron con el incremento en CPOD.

Key words: DMFT index, colony forming units, *Streptococcus*, *Lactobacillus*.
Palabras clave: Índice CPOD, unidades formadoras de colonias, *Streptococcus*, *Lactobacillus*.

INTRODUCTION

In our days, dental caries represents the most common oral disease in mankind. WHO considers that 60 to 90% of all population is afflicted. Changes in hygiene and eating habits have caused an increase in caries prevalence in different populations.[1] Caries is generated by the interaction between the host and microorganisms which develop in favorable environments. The direct relationship found between presence of microorganisms and caries prevalence, the infectious nature of this condition as well as its recognition, isolation and identification of germs' specific characteristics, allow the determination of risks of developing caries, as well as their severity or progress stage.

Streptococcus mutans is classified into two groups: *Streptococcus viridians* and *Streptococcus* non.[2] In human beings, main serotypes are c/e/f and d/g which respectively form species of *Streptococcus mutans* and *Streptococcus sobrinus*.[3] Many studies have been conducted on the positive co-relation between caries prevalence and *Streptococcus mutans* and

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Lactobacillus.[4,5] There is a great risk of developing caries when these microorganisms are found in high counts. Other studies showed that *Lactobacillus* and *Streptococcus mutans* are found in great numbers of subjects afflicted with dental caries.[6-10]

Dental caries has been considered a social issue; it is a multi-factorial disease influenced by eating habits, time, drinking water consumption, hygiene, educational level and access to health services. In permanent dentition, caries appears after eruption of the lower first molar, when subjects are approximately six years old; pits and fissures are the most affected areas.

Caries lesions are easily detected during a clinical exam along with inter-proximal X-rays. Nevertheless these proving facts do not predict caries activity or indicate a patient's susceptibility to suffering this condition.

The objective of this research project was to determine relationship existing between count of *Lactobacillus* and *Streptococcus mutans* with caries presence, as well as with caries susceptibility exhibited by first year students enrolled at the National School of Dentistry, National University of Mexico (UNAM) in the scholastic year 2012-2013.

MATERIAL AND METHODS

Study population

In the present study, population was composed of 380 first year students enrolled at the National School of Dentistry, National University of Mexico, during 2012-2013 scholastic period.

Dental assessment

Dental assessment was conducted according to World Health Organization recommended guidelines. In order to measure coronary caries, DMFT indexes were obtained as well as their Klein and Palmer components. Data collection was performed by two dentists: one conducted clinical examinations and the other collected data. Both presented optimum level of calibration and criteria, techniques and procedures homogeneity. Examiners' diagnosis criterion reliability (intra-calibration) was above 90%.

Examinations were conducted with a halogen frontal light lamp, using real-size mirrors and dental explorers. All patients received information on the test as well as oral hygiene instructions. Assessment of all teeth was initiated at the upper right quadrant, proceeding then to the upper left quadrant, then lower left quadrant and completing examination with the lower right quadrant.

Saliva collection

Saliva samples were collected during the first hours of the morning, before brushing or food intake. A paraffin stimulation (0.9 g) for 5 minutes was conducted, until achieving collection of 5 mL saliva, samples were homogenized and placed in a ice bath, 400 μL saliva were taken and diluted in saline phosphate buffer (1:10). A second 1:100 dilution in 7.4 saline phosphate buffer of the first dilution was executed; 100 μL were taken to inoculate the culture medium. Samples were processed immediately after collection.

Count of saliva caries-associated microorganisms

For *Streptococcus mutans* count, 100 μL of the 1:100 dilution were taken to be later inoculated in mitis salivarius agar (Difco Labs, Detroit, Mich USA) supplemented with bacitracin (2 units/mL) and saccharose (20%) (Sigma Chemical Co. St Louis, Mo, USA) (MSB). For *Lactobacillus* count, 100 μL of the 1:100 solution were taken to be later inoculated into Rogosa agar (Difco Labs, Detroit, Mich USA) supplemented with glacial acetic acid (1.32 mL/L) (Sigman Chemical Co. St Louis, Mo USA) at a 5.4 final pH. Media were incubated at 37 °C in anaerobiosis jars for 72 hours. Once this time had elapsed, count was made of *Streptococcus mutans* and *Lactobacillus* (UFC/mL) colony forming units. They were divided into two classes: $< 10^4$ and $> 10^4$ of *Lactobacillus* or *Streptococcus* per mL of saliva.

Results analysis

Mean (± SD) of DMFT indexes and their components were calculated. Data were compared using non parametric methods such as χ^2 test using SPSSPC software (SPSS Inc, Chicago, Ill, USA).

RESULTS

Risk factors are attributes or characteristics which provide the individual with a certain degree of propensity to contract a disease. Since it constitutes a measurable probability, it possesses predictive values and can be used to advantage in individual, group or community prevention processes.

Studied population consisted of 380 students (278 females and 102 males). Mean age was 19.50 ± 1.63. Mean age in females was 19.49 ± 1.71 and in males 18.96 ± 1.59. Minimum age was 17 years (n = 2) and maximum age was 36 years (n = 1).

Results showed that DMFT index mean was 7.25 ± 0.019; no significant differences were found between males and females, with 7.11 ± 0.02 and 7.29 ± 0.07 respectively. Students with zero DMFT were 34; 20 year old population was the most affected, showing 7.51 DMFT index and 3.24 average of caries-affected teeth (SD ± 0.008) *(Table I)*.

Out of all examined students, 26.84% were male and 73.15% female. Caries average in males was (3.16 ± 0.03) above females' average (3.27 ± 0.01). Moreover, males exhibited larger numbers of filled teeth (4.09 ± 0.04), which would suggest males attended dental visits more frequently *(Table II)*.

Indexes of caries due to carbohydrate intake did not show significant increase of lesions; DMFT index only increased (1.17 times) with respect to carbohydrate intake *(Table III).*

With respect to relationship between caries index and pits and fissures treatment *(Table IV)*, results suggested that population which received this treatment exhibited greater DMFT indexes (7.31 ± 0.05). Differences were found in the average of teeth with caries with respect to this treatment.

With respect to presence of *Streptococcus mutans* and *Lactobacillus* in the present study, it can be said that out of the 380 students, 95.78 exhibited *Streptococcus mutans* in their saliva and 52.76 exhibited *Lactobacillus*. With respect to distribution of DMFT index with number of *Streptococcus* and *Lactobacillus* colony-forming units *(Table V)* it was found that caries average increased in relation to increase of colony-forming units for *Streptococcus mutans*, which differed from *Lactobacillus* colony-forming units. Nevertheless, DMFT mean index increased with respect to the number of colony-forming units for both microorganisms. Correlation coefficient between number of *Streptococcus* and *Lactobacillus* was 0.5 (Spearmen range of correlation test with $p < 0.001$).

DISCUSSION

Caries indexes in developing countries populations are higher than those observed in developed countries.[6,7] High caries incidence in the population is especially deplorable, when one considers that caries is a preventable disease as proven by different programs applied for decades in different countries of the world.[8]

The present research cannot be compared to any other since this is the first project undertaken to ascertain caries and cariogenic microorganisms incidence in the community of students entering our National School of Dentistry. The present study can

Table I. Caries index according to age.

Age	N	Caries	Lost	Filled	DMFT
18	164	3.34 ± 0.020	0.44 ± 0.002	3.37 ± 0.02	7.17 ± 0.04
19	125	3.08 ± 0.02	0.4 ± 0.0003	3.71 ± 0.02	7.2 ± 0.05
20	36	3.31 ± 0.09	0.20 ± 0.005	4 ± 0.11	7.51 ± 0.20
Total	380	3.24 ± 0.008	0.37 ± 0.0009	3.63 ± 0.009	7.25 ± 0.019

Table II. Caries index according to gender.

Gender	N	Caries	Lost	Filled	DMFT
Males	102	3.167 ± 0.03	0.46 ± 0.33	4.09 ± 0.04	7.25 ± 0.07
Females	278	3.273 ± 0.01	0.338 ± 0.001	3.464 ± 0.01	7.076 ± 0.02

Table III. Caries index according to sweets intake.

Number of sweets	N	Caries	Lost	Filled	DMFT
0	212	3.15 ± 0.01	0.30 ± 0.001	3.60 ± 0.01	7.06 ± 0.03
1.00	140	3.28 ± 0.02	0.42 ± 0.003	3.56 ± 0.02	7.27 ± 0.051
2.00 o más	28	3.71 ± 0.13	0.60 ± 0.02	4.21 ± 0.15	8.53 ± 0.30
Total	380	3.24 ± 0.008	0.37 ± 0.000	3.63 ± 0.009	7.25 ± 0.01

Table IV. Caries index according to pits and fissures preventive treatments.

Pits and fissures treatment	N	Caries	Lost	Filled	DMFT
Yes	142	3.51 ± 0.024	0.30 ± 0.002	3.48 ± 0.024	7.31 ± 0.05
No	238	3.08 ± 0.012	0.41 ± 0.001	3.71 ± 0.01	7.21 ± 0.03
Total	380	3.24 ± 0.00	0.37 ± 0.009	3.63 ± 0.009	7.25 ± 0.01

Table V. DMFT index per colony forming units for *Streptococcus* and *Lactobacillus*.

	Streptococcus				
Colony forming units	N	Caries	Lost	Filled	DMFT
< 10^4	95	2.65 ± 0.02	0.43 ± 0.004	4.02 ± 0.04	7.12 ± 0.07
> 10^4	285	4.25 ± 0.01	0.25 ± 0.009	2.95 ± 0.01	7.47 ± 0.02
Total	380	3.24 ± 0.008	0.37 ± 0.009	3.63 ± 0.009	7.25 ± 0.01
	Lactobacillus				
Colony forming units	N	Caries	Lost	Filled	DMFT
< 10^4	181	3.04 ± 0.01	0.33 ± 0.001	2.82 ± 0.01	6.20 ± 0.03
> 10^4	199	3.42 ± 0.01	0.40 ± 0.002	4.36 ± 0.02	8.19 ± 0.04
Total	380	3.24 ± 0.008	0.37 ± 0.009	3.63 ± 0.009	7.25 ± 0.01

be considered of the utmost importance, since it provides knowledge of their oral health. Moreover, great disparities have been found in DMFT index in different populations in Mexico, this is especially due to differences in socioeconomic status.[9]

First molars are the most caries-affected teeth. Since permanent first molars are the first to erupt; most lesions are found in pits and fissures. Information here gathered was consistent with caries patterns revealed in most epidemiologic studies. Differing from developed countries populations DMFT's most important caries component is the filled tooth.[10]

In the present project it was also found that state of microorganism presence is correlated to dental caries. We equally found that there is a high correlation among *Lactobacillus* and *Streptococcus* colony forming units with high DMFT indexes. A correlation among *Streptococcus* with DMFT index was equally found, this can reflect high consumption levels of saccharose.[11]

CONCLUSION

To summarize, it can be proposed that this study might be the basis to observe whether changes are effected in the circumstances of microorganisms existing in a group of subjects who will receive professional training along their studied field of Dentistry, so as to be able to create a predictive model in the future.

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