

Relationship between Severe Early Childhood Caries, Mother's Oral Health and *Mutans Streptococci* in a Low-Income Group: Changes from 1996 to 2007

Ma. Esther Irigoyen Camacho* / Leonor Sánchez Pérez** / Álvaro García Pérez *** / Marco Antonio Zepeda Zepeda****

The relationship between dental caries and mutans streptococci (ms) in children and their mothers is a key factor in the prevention of early childhood caries. Changes in early childhood caries over time in Mexican children have not been well documented. Objective: to compare the dental caries indices and presence of ms in 3- to 5-year-old children and their mothers in two surveys, the first one carried out in 1996 and the second one in 2007. Study design: cross-sectional study. Results: The first survey included 102 mother-child dyads and the second 103. Dental caries index dmfs was 7.17 (± 10.9) and 4.58 (± 7.1) in the first and second survey, respectively ($p < 0.05$). Ms levels were higher in 1996 than in 2007 ($p < 0.001$). The prevalence of Severe Early Childhood Caries (SECC) was 42.2% and 34.95% in first and second surveys, respectively, ($p > 0.05$). Children with SECC showed higher counts of ms than children without this condition. A high caries index in mothers was associated with higher caries experience in their children. Conclusion: the comparisons of the surveys indicated a decline in dental caries indices over an 11-year period. High caries experience in the mother had a negative impact on the oral condition of the child.

Keywords: early childhood caries, mother-child pairs, mutans streptococci, low income district, dental caries trends.

J Clin Pediatr Dent 33(3): 241–246, 2009

INTRODUCTION

In numerous populations around the world, schoolchildren and adolescents have experienced a decline in dental caries;¹ however, some countries are showing a halt in this decline.^{2,3} Different trends in preschool children have been found: while a decrease in dental caries in some

preschool children in Italy has been observed, children aged 2–5-years-old in the United States have suffered an increase in rates of caries.^{4,5} In Mexico, state and local surveys have shown a trend towards a decrease in prevalence and severity of this disease in school children.^{6,7} However, the frequency of dental caries is still high, particularly in central Mexico.⁸ A National Salt Fluoridation Program started in Mexico in the early nineties; by law, table salt manufacturers add 200 to 250 F mg/Kg of salt.⁹ Prior to the salt fluoridation program a survey selecting a representative sample of kindergarten and elementary school children was performed in Mexico City. This survey identified a high caries index in five-year-old children, with a mean dmft of 5.25 and a dmfs of 10.65.¹⁰ At present, there is not sufficient data to allow the identification of dental caries trends of preschool children in the country, or to assess the impact of the different preventive measurements, such as fluoride dentifrices and the salt fluoridation program.

The study of dental caries in the primary dentition is important not only for the resulting deterioration in the quality of life of young children, but also because dental caries in the primary dentition is one of the best predictors of caries in the permanent dentition. Studies in preschool children in several countries have shown that the presence of dental caries in the primary molars is the best indicator of future caries development in the permanent teeth.^{11,12} Another risk

* Ma. Esther Irigoyen Camacho, DDS, MPH, DO, Division of Biological Sciences and Health, Department of Health Care. Metropolitan Autonomous University Xochimilco.

** Leonor Sánchez Pérez, DDS DO, Division of Biological Sciences and Health, Department of Health Care. Metropolitan Autonomous University Xochimilco.

*** Álvaro García Pérez, DDS, Division of Biological Sciences and Health, Department of Health Care. Metropolitan Autonomous University Xochimilco.

**** Marco Antonio Zepeda Zepeda, Phys., Division of Biological Sciences and Health, Department of Health Care. Metropolitan Autonomous University Xochimilco.

Send all correspondence to:

Dra. María Esther Irigoyen-Camacho, CD, MPH, DO, Metropolitan Autonomous University Xochimilco, Calzada del Hueso 1100, Col Villa Quietud, CP 04960, México D. F. México.

Phone: +525 54837530.

E-mail: meirigo@correo.xoc.uam.mx

factor for dental caries is the presence of *mutans streptococci* (*ms*) in the oral cavity; high counts of these bacteria are associated with a high incidence of dental caries.¹³ Mexican studies that detected the frequency of *ms* have mainly involved school children and there is little information about younger age groups. A study carried out in Mexico City in 12–14-year-old city children and indigenous children showed that 95% of the children in the City had *ms* while 75% indigenous children presented it.¹⁴ Another Mexico City study found a prevalence of 80% caries rate in 8-10-year-old children and more than half of these children had a high *ms* level counts ($\geq 10^5$ mutans counts).¹⁵ In 10–13-year-old children from the State of Zacatecas the prevalence of *ms* was 81.4%.¹⁶

Vertical transmission of *ms* frequently occurs from the mother to the child. Different longitudinal studies, in which genotyping was performed, have shown that most of the children share the *ms* harbored in their mother's oral flora.^{17,18} *Ms* was detected in a study carried out in Mexico City involving mother and child pairs. In children less than 1-year-old the prevalence of *ms* was 31% and in the 1–2-year-old group 50% of the children harbored this microorganism; none of the children examined showed cavitated caries lesions.¹⁹ The lack of information on the frequency at which *ms* is found in preschool children limits the identification of changes in the occurrence of these microorganisms and the modifications related to preventive programs. The presence of high caries experience in children has been associated with high dental caries in their mothers.^{13, 20} The understanding of these kinds of relationships is significant from a public health perspective and in clinical practice, favoring timely interventions that could decrease the risk of early childhood caries. This is of particular importance in low-income groups given that they share a larger burden of oral diseases than their higher income counterparts.²¹

The aim of the present study was to compare the dental caries indices, prevalence of severe early childhood caries (SECC) and presence of *ms* in 3–5-year-old children and their mothers in two surveys, the first one carried out in 1996 and the second one in 2007, in a low-income area of Mexico City.

MATERIALS AND METHODS

The first survey was carried out in 1996 and the second in 2007 at two main day care centers located in south-east District of Mexico City. The day care centers are located in a low-income area of Mexico City.²² Meetings with the health and school authorities were held in order to present the project and to obtain permission to complete the study. The aims and procedures of the study were explained to the parents and they were asked to sign a consent form. In the first survey a group of 111 mothers was invited to participate in the study and the non-response rate was 8.1% (9), and in the second survey a group of 113 mothers was invited to participate in the study and the non-response rate was 8.8% (10). Mothers that refused to participate in the study indicated lack of time to undergo the oral examination and to provide salivary

samples, whilst a further obstacle was that some mothers did not take their child to school or collect them.

The study protocol was reviewed by the Research Committee of the Division of the Biological and Health Sciences of the Metropolitan Autonomous University and the technical and ethical aspects were considered. The protocol was also put for consideration to the district health and school authorities; logistic and ethical aspects were discussed and their support was given.

The World Health Organization (WHO) criteria were followed for the detection of dental caries.²³ WHO probe and plain mirrors were used. The exams were carried out with an auxiliary light. No radiographs were taken. Additionally, in the 2007 survey non-cavitated caries lesions were identified based on the incipient caries lesions criteria of Ismail.²⁴ Before the exams the tooth surface was cleaned with wet sterile gauze pads, then the tooth was examined to detect spots in the enamel surface indicating changes in color (white or brown) related to enamel demineralization; after that, the tooth was air dried to detect white/brown-spot lesions. When in doubt about the presence of a cavity the WHO probe was carefully used to identify whether the instrument penetrated the tooth. Localized breakdown of the enamel without going into dentin was considered an incipient lesion. In this case the surface was classified as a d₁ lesion.^{24,25} When the base or lateral walls of the cavity were soft, the surface was classified as a cavitated lesion which conformed the decayed component of dmfs index.²³ The oral examinations were performed by the same dental specialist in both surveys. In the first survey Kappa=0.87 and in the second Kappa=0.88 for the presence of dental caries at the tooth level using the WHO criteria, and Kappa=0.81 for incipient lesions. Severe Early Childhood Caries (SECC) was classified according to the American Academy of Pediatric Dentistry definition.²⁶

Bacteriological procedures

In the first survey, the levels of *mutans streptococci* in saliva were measured using the commercial Dentocult SM strip *mutans* Test (Orion Diagnostica, Espoo, Finland). The test vials were placed upright and were incubated at 37 °C for 48 h. The density of bacteria was evaluated according to the manufacturer's chart. In the second survey the levels of *ms* were assessed using the CRT bacteria kit (Ivoclar Vivadent, Schaan, Liechtenstein). The slides were inoculated with saliva obtained by chewing a paraffin pellet. The test vials were placed upright and were incubated at 37 °C for 48 h. Bacteria levels were evaluated by comparing the density of colonies with the standard of the manufacturer's chart. In children who had used antibiotics recently, collection of the salivary sample was postponed for 3 months.

Statistical analysis

Data analysis was carried out with JMP software 6.0 (SAS Institute Inc, Cary NC, USA). Bivariate analysis was performed using Student's t-test, the Chi-squared statistic and Spearman's rank correlation coefficient (r). The levels of

ms were dichotomized based on the manufacturer's chart. A low level was defined as $ms < 10^5$ CFU/ml saliva and a high level as $ms \geq 10^5$ CFU/ml saliva. The Restorative Treatment Needs index was calculated as follows: $[d/(d+f)](100)$ based on the dmft index. Multiple linear regression models were fitted using the dental caries index as the dependent variable; for the second survey, incipient lesions were also included (d_1 mfs). Possible interactions were tested in all cases. The level of statistical significance was set at $p < 0.05$. The data from the two day care centers were analyzed together since no significant differences were detected in the main variables studied.

RESULTS

First survey results

In the first survey (1996), 102 child and mother dyads were included. The mean age of the participating children was 53.5 months (± 8.71): 33.3% were 3 years-old, 45.1% 4 years-old and 21.6% were 5 years-old. The percentage of girls was 44.12% (45).

The mean dmft was 4.03 (± 3.74): in the 3-year-olds it was 3.53 (± 3.10), in the 4-year-olds 3.92 (± 3.82) and in the 5-year-olds 4.92 (± 4.29). Table 1 presents dmfs index and its

Table 1. Dental caries index (dmfs) and its components by age group in the 1996 and 2007 surveys

age	YEAR 1996			
	decayed	missing	filled	dmfs
	Mean (Std Dev)	Mean (Std Dev)	Mean (Std Dev)	Mean (Std Dev)
3	3.03 (2.77)	0.00 (0.00)	1.53 (4.90)	4.56 (7.67)
4	3.79 (4.53)	0.27 (1.40)	2.46 (6.13)	6.51 (12.06)
5	4.00 (3.57)	0.40 (1.05)	6.27 (13.42)	10.67 (18.04)
3-5	3.75 (4.02)	0.33 (1.43)	3.10 (8.30)	7.17 (10.90)
age	YEAR 2007			
	decayed	missing	filled	dmfs
	Mean (Std Dev)	Mean (Std Dev)	Mean (Std Dev)	Mean (Std Dev)
3	1.03 (2.13)	0.00 (0.00)	2.37 (7.28)	3.40 (9.41)
4	1.89 (2.52)	0.00 (0.00)	2.55 (5.36)	4.44 (7.88)
5	2.85 (3.78)	0.00 (0.00)	2.74 (6.08)	5.59 (9.86)
3-5	2.01 (3.00)	0.00 (0.00)	2.57 (6.11)	4.58 (7.10)

Comparison of 1996 with 2007: ds ($p < 0.001$), ms ($p < 0.025$), fs ($p > 0.05$), dmfs ($p < 0.049$).

Table 2. Dental caries indexes and its components of children and mothers examined in the 1996 and 2007 surveys

	YEAR 1996				YEAR 2007			
	Children		Mothers		Children		Mothers	
	index	mean (sd)	Index	mean (sd)	Index	mean (sd)	Index	mean (sd)
dt	2.99	(2.52)	DT	5.21 (3.52)	dt	1.39 (1.84)	DT	3.98 (3.27)
mt	0.11	(0.47)	MT	1.88 (2.23)	mt	0.00 (0.00)	MT	1.97 (2.22)
ft	0.93	(1.99)	FT	6.02 (4.82)	ft	1.29 (2.29)	FT	6.21 (5.31)
dmft	4.03	(3.74)	DMFT	13.11 (5.07)	dmft	2.68 (2.91)	DMFT	12.16 (4.29)

(sd) Standard deviation. Comparison of 1996 with 2007: dt ($p < 0.001$), mt ($p < 0.024$), ft ($p > 0.05$), dmft ($p < 0.008$), DT ($p = 0.019$), MT ($p > 0.05$), FT ($p > 0.05$), DMFT ($p > 0.05$).

components across age groups. The decayed teeth comprised the largest part of the dmft index (74.19%), followed by the filled teeth component, which accounted for 23.08% of the index, and the smallest fraction derived from the extracted teeth, 2.73%. Treatment needs were high among these children at 77.4%. 16.7% of the children showed a dmft score of zero, whilst caries prevalence was 83.3%. None of the children had dental sealants. Severe Early Childhood Caries (SECC) was found in 43 (42.2%) children: 8 (23.5%) of the 3-year-olds, 21 (45.7%) of the 4-year-olds and 14 (63.6%) of the 5-year-olds experienced this level of dental disease. The bacterial culture showed that 73.5% (75) of the children had high *ms* counts ($\geq 10^5$ CFU/ml saliva). No bacterial growth was observed in only 2 (1.96%) salivary samples.

The mean age of the mothers was 31.28 (± 4.14) years old. Table 2 presents their mean DMFT and its components. The restorative treatment needs index was 46.1%. In this group of mothers none had a zero DMFT score. The bacterial culture identified 46.09% of mothers with high *ms* counts ($\geq 10^5$ CFU/ml). No *ms* growth was observed in 3 (2.94%) saliva samples.

The statistical test for the association between the children's dmft index and the level of *ms* failed to show any significant association: the dmft for the low level *ms* group was 3.90 (± 5.29) and for the high level group it was 4.16 (± 3.46) ($p > 0.05$); however, the decayed component was associated with *ms* level: children with low counts had a mean dt=2.48 (± 2.71) and high counts dt=3.51 (± 2.4), $p = 0.016$, controlling by age. The children whose mothers showed low levels of *ms* had a mean dmft=3.80 (± 3.98) and those with mothers harboring high levels of bacteria had a dmft=4.26 (± 3.82), ($p > 0.05$). There was a positive correlation between the dmft of the children and the DMFT of the mothers ($r = 0.2993$, $p = 0.008$). Mothers harboring low levels of *ms* had a lower mean DMFT index (11.69 \pm 4.60) than those presenting with high levels of *ms* (14.53 \pm 5.22) ($p = 0.012$).

A multiple regression model was constructed using the children's caries index as the dependent variable and the children's age and mothers' DMFT as independent variables. The estimates were: age $\beta = 0.232$ ($p = 0.108$) and mother's DMFT $\beta = 0.094$ ($p = 0.006$). The results showed that mothers with a higher caries index had children with a higher caries index.

Second survey results

The second survey (2007) included 103 dyads. The mean age of the children was 53.71 (± 11.31) months: 28 (27.18%) were 3 years-old, 39 (37.86%) 4 years-old and 36 (34.95%) were 5 years-old. The percentage of girls was 48.5% (50).

The mean dmft index was 2.68 (± 2.91). Table 2 presents the components of the dmft index; no extracted teeth were detected in the oral examination of the children. The decayed component comprised 58.9% of the index. 35.9% (37) of the children showed a dmft index of zero. The mean number of incipient lesions was 7.27 (± 4.11), and d_1mfs was 12.85 (± 9.68). The proportion of children without caries lesions including the incipient stage was 1.9%. The mean restorative treatment needs index was 51.81%. Only 3.9% of the children had dental sealants. The dmfs index across the age groups is presented in Table 1. The SECC prevalence was 34.95% (36): in 3-year-olds it was 28.57% (8), in 4 year-olds 33.33% (13) and 5 year-olds 41.67% (15). The bacterial cultures showed that 31.1% (32) of the children had high *ms* counts ($\geq 10^5$ CFU/ml saliva). No bacterial growth was observed in 6 (5.83%) salivary samples.

In the second survey, the mean age of the mothers was 32.46 years-old (± 5.93), and their mean DMFT was 12.16 (± 5.30), the components of the index are presented in Table 2. The mean restorative treatment needs index was 39.01%. The salivary cultures showed that 45.63% had high counts of *ms*. Only 3 (2.9%) women did not show a positive culture.

There was no significant association between the dmft index and its components and the children's *ms* level: the dmft for low *ms* counts was 2.28 (± 2.83) and for high counts it was 3.00 (± 3.18) ($p > 0.05$). The SECC showed an association with children's *ms* level: 29.03% of the low level *ms* group had SECC whilst in the group with a high *ms* level 50% experienced this condition ($p = 0.035$). No significant association was found between mothers' *ms* level and children's caries index: 2.47 (± 2.99) for low mothers' *ms* level and 2.98 (± 2.96) for high mothers' *ms* level ($p > 0.05$). However, children's incipient lesions were associated with the level of *ms* in the mother, ($p = 0.016$), controlling by age. The DMFS of the mothers correlated with the dmfs of the children ($r = 0.2130$, $p = 0.033$).

A multiple regression model was fitted using the dmfs index as the dependent variable and the children's age, *ms* and mothers' DMFS as independent variables. The estimates calculated in the model are shown in Table 3. The effect of

Table 3. Multiple linear regression model results fitting caries index (dmfs) of the children of the 2007 survey

Variable	Estimate	Standard error	P
Age (months)	0.141	0.069	0.045
MS (children,0)	-0.552	0.801	0.492
Age * MS (children)	-0.203	0.071	0.005
DMFS (mother)	0.103	0.733	0.044
Intercept	-5.394	4.101	0.192

MS :Mutans Streptococci, 0 = CFU $< 10^5$, $R^2 = 0.1695$ $p = 0.002$

ms level on dmfs index was different by age group ($p = 0.005$); 4-year-old children with a low *ms* level had a dmfs = 3.63 and their counterparts with a high *ms* level had a dmfs = 4.80 (the difference was 1.17), while for 5-year-old children the dmfs = 3.05 for low *ms* level and dmfs = 9.21 for high *ms* level (difference 6.16). A further analysis including early caries lesions (d_1mfs) indicated that the same variables were statistically significant.

Comparison of the 1996 and the 2007 surveys

No significant difference in the age and sex distribution of the children was found between the two surveys ($p > 0.05$). The difference in the children's dmft index in 1996 and 2007 was 1.35, corresponding to a reduction in dental caries index close to 33.5%, ($p = 0.008$), (Table 2). Table 1 presents the dmfs index for 1996 and 2007; a significant decrease in this index was detected ($p < 0.05$). The decayed component was significantly lower ($p < 0.001$), there were no missing teeth in 2007, and no significant difference was detected in the filled component of the index ($p > 0.05$). The proportion of children with a dmft index of zero was 20% lower in 2007 (35.9%) than in 1996 (16.7%) ($p = 0.002$). Thus, caries prevalence was 23.05% lower in 2007 (64.1%) than in 1996 (83.3%).

Children with a 100% treatment needs index accounted for about 32.0% in 2007 while in 1996 this percentage was 70.59% ($p < 0.001$). A lower proportion of children with a high *ms* level was found in the 2007 survey compared with the 1996 salivary samples ($p < 0.001$). The proportion of children experiencing SECC was similar in both studies ($p > 0.05$).

None of the mothers presented a DMFT = 0 in either survey. The data from the two surveys showed a large DMFT index in these women, and no significant difference was detected between the surveys ($p > 0.05$). However, a difference was found in the composition of the index: in 2007 mothers exhibited 23% less decayed teeth than those examined in the first survey ($p = 0.019$), (Table 2). The proportion of women with high *ms* counts was similar in both surveys ($p > 0.05$).

DISCUSSION

The results of the oral examination of the 1996 children compared with those examined in 2007 indicated a reduction in dental caries prevalence and severity. The caries index trend towards reduction appears to be moderate given the 33% decrease over a period of 11 years. The data from the City survey carried out before the initiation of the Salt Fluoridation Program showed higher dental indices than the values obtained in 1996 (5.25 vs. 4.03).¹⁰ There are some more recent local studies involving Mexican preschool children. A study carried out in a southern state (Campeche) suggested a lower caries index (dmft = 1.4) compared with the values of the present study.²⁷ Lower indices were also found in a study carried out in Mexico City in a day care center for children of federal government employees (dmft = 0.73).²⁸ It is possible that the low family income of the children involved

in the present study favored higher caries indices than the scores detected among preschool children of parents working for the federal government. The children participating in the present study live in a district where more than 75% of the population earns less than two minimal wages.²²

A lower proportion of the children examined in 2007 showed high counts of *ms* compared with the 1996 children. Several caries preventive regimes have shown a reduction in *ms* counts accompanied with a decline in new caries lesions.¹³

Despite the reduction in the dental caries index, more than a third of the children had SECC in 2007. This was associated with the level of infection by *ms* found in these preschool children, such that half of the children with high *ms* counts had SECC. In the 2007 survey incipient lesions were recorded and caries prevalence including this type of lesion was around 98%. The detection of this type of lesion provides the opportunity for early intervention that will benefit the children.

The presence of a high prevalence of dental caries and the numerous preschool children showing SECC identified in the study were the consequence of the predominance of the caries risk factors over the protective factors. Fluoride is recognized as a key protective factor. In Mexico the National Salt Fluoridation Program has experienced some difficulties, particularly those concerned with securing the appropriate fluoride levels in the table salt.^{29,30} A recent study showed that the mean fluoride concentration in the table salt packages was around 120 ppm.³¹ This concentration is about half of the quantity officially approved for the Health Ministry in Mexico.⁹

The models fitted in each survey identified an association between the caries index of the children and the caries experience of the mother. It is possible that this association relates to several factors shared by mother-child dyads, such as high sucrose diets, frequent food consumption, and oral hygiene practices as well as genetic factors that alter caries risk. In addition, the model for the 2007 data identified an interaction between age and *ms*. Differences in the effect of *ms* level on the caries index increased with age. As pointed out in the classic model proposed by Newbrun, time is a factor modulating the development of dental caries,³² and preventive measures probably delay the development of caries lesions in these children. Also it is possible that older children harbor a more aggressive biofilm. Mexican 8-10-year-old children showed a 40.0 % prevalence of *Streptococcus mutans* and 56.7% of *Streptococcus sobrinus*.¹⁵ It has been found that the simultaneous presence of *Streptococcus sobrinus* and *Streptococcus mutans* increases the risk of dental caries compared with only harboring *Streptococcus mutans*.³³ *Streptococcus sobrinus* seems to appear later in the oral cavity than *Streptococcus mutans*.¹⁷

A positive finding from the comparison of the two surveys was a reduction in treatment needs among the preschool children evaluated. Accordingly, there has been an increase in utilization of dental services, which in conjunc-

tion with the higher availability of fluoride, through the dentifrices and table salt, may explain some of the reduction in caries incidence observed in these preschool children.

Mothers showed no significant difference in the mean number of extracted or filled teeth in the two surveys; however a lower value for the decayed component of the DMFT index was observed in the second survey. Despite this reduction, the mothers' dental caries index and restorative treatment needs remained at a high level.

Treatment needs is still a problem for this population, as some of the children exhibit extensive damage that would require treatment, not easily available for these low-income children. Moreover, very few dental sealants are applied in these groups. According to the American Dental Association, sealants should be placed on the primary molars of children who are susceptible to dental caries³⁴; many of the children in this population fulfill this criterion.

The association between the dental caries experience in the mother and the child points to the need to consider the mother's oral condition as a key factor in young children's oral health. In addition, despite the observed reduction in caries, there is still a high proportion of children with severe ECC. In view of this scenario, an early caries prevention program involving mother-child dyads is required in this population group.

CONCLUSION

This study showed a trend towards a decrease in dental caries prevalence and severity over an 11-year interval in preschool children from a low-income area. However, there was still a high prevalence and severity of dental caries. The high caries experience of the mothers was associated with greater deterioration of the oral health of the child. Public health dentists and clinicians should consider this information to design preventive interventions and in treatment planning for young children.

ACKNOWLEDGEMENTS

This research project was funded by the National Council of Science and Technology (CONACYT, project number: S52823-M) and the Metropolitan Autonomous University (UAM-X, project number: 211535114). The authors wish to thank the personnel at the day care centers, and the mothers and the children who kindly agreed to participate in this project.

REFERENCES

1. Newbrun E. Dental caries in the future: a global view. *Proc Finn Dent Soc*, 88: 155–161, 1992.
2. Pitts NB, Chestnutt IG, Evans D, White D, Chadwick B, Steele JG. The dental caries experience of children in the United Kingdom, 2003. *Br Dent J*, 200: 313–320, 2006.
3. Armfield JM, Spencer AJ. Quarter of a century of change: caries experience in Australian children, 1977–2002. *Aust Dent J*, 53: 151–159, 2008.
4. Ferro R, Besostri A, Oliveri A, Stellini E, Mazzoleni S. Preschoolers' dental caries experience and its trend over 20 years in a North-East Italian Health District. *Eur J Paediatr Dent*, 8: 199–204, 2007.

5. Dye BA, Tan S, Smith V, Lewis BG, Barker LK, Thornton-Evans G, Eke PI, Beltán-Aguilar ED, Horowitz AM, Li CH. Trends in oral health status: United States, 1988–1994 and 1999–2004. *Vital Health Stat* 248: 1–92, 2007.
6. Zamudio-Gómez MA, Gaitán-Cepeda LA, Bravo-Pérez M, Gómez-Llanos H. Impacto a corto plazo de la cartilla de salud bucal sobre la prevalencia de caries en niños de Tijuana, Baja California, México. [Short-term impact of the oral health card on the prevalence of dental caries in children in Tijuana, Baja California, Mexico] *Rev Odontol Mex*, 9: 137–140, 2005.
7. Velázquez-Monroy O, Vera-Hermosillo H, Irigoyen-Camacho ME, Mejía-González A, Sánchez-Pérez L. Changes in the prevalence of dental caries in schoolchildren in three regions of Mexico: surveys from 1987–1988 and 1997–1998. *Pan American Journal of Public Health*, 13: 320–326, 2003.
8. Secretaría de Salud, Dirección General de Medicina Preventiva, Subdirección de Salud Bucal. Encuesta Nacional de Caries Dental. [Ministry of Health, Preventive Medicine Main Office, Department of Oral Health. National Survey of Dental Caries] México, D.F.: SSA, 1997–2001.
9. Norma Oficial Mexicana NOM-040-SSA1-1993. Bienes y Servicios. Sal yodada. Sal fluorurada. Requisitos sanitarios. México D.F.: Diario Oficial de la Federación; 23 de Marzo de 1995.
10. Irigoyen ME, Sánchez L, Zepeda MA. Prevalencia y severidad de caries en dientes primarios en alumnos de jardines de niños y escuelas primarias del Distrito Federal. [Prevalence and severity of tooth decay in primary teeth in kindergarten and elementary schoolchildren in the Federal District.] *PO*, 19: 23–30, 1997.
11. Skeie MS, Raadal M, Strand GV, Espelid I. The relationship between caries in the primary dentition at 5 years of age and permanent dentition at 10 years of age – a longitudinal study. *Int J Paediatric Dent*, 16: 152–160, 2006.
12. Li Y, Wang W. Predicting caries in permanent teeth from caries in primary teeth: an eight-year cohort study. *J Dent Res*, 81: 561–566, 2002.
13. Tanzer JM, Livingston J, Thompson AM. The microbiology of primary dental caries in humans. *J Dent Educ*, 65: 1028–1037, 2001.
14. Del Rio Gomez I. Dental caries and mutans streptococci in selected groups of urban and native Indian schoolchildren in Mexico. *Community Dent Oral Epidemiol*, 19: 98–100, 1991.
15. Sánchez-Pérez L, Acosta-Gio E. Caries risk assessment from dental plaque and salivary *Streptococcus mutans* counts on two culture media. *Archives Oral Biol*, 46: 49–55, 2001.
16. Aguilera GLA, Padilla P, Aguilar R, Frausto S, Aceves MC, Enriquez EA. Niveles de *Streptococcus mutans* y prevalencia de caries dental en una población de escolares de la zona urbana de la ciudad de Zacatecas. [Levels of *Streptococcus mutans* and prevalence of dental caries in a population of schoolchildren of the urban area of the city of Zacatecas] *Rev Asoc Dent Mex LXI*: 85–91, 2004.
17. Klein MK, Flório F, Pereira AC, Hofling JF, Goncalves BR. Longitudinal study of transmission, diversity and stability of *Streptococcus mutans* and *Streptococcus sobrinus* genotypes in Brazilian nursery children. *J Clin Microbiol*, 42: 4620–4626, 2004.
18. Lindquist B, Emilson CG. Colonization of *Streptococcus mutans* and *Streptococcus sobrinus* genotypes and caries development in children to mothers harboring both species. *Car Res*, 38: 95–103, 2004.
19. Revuelta Pérez R, Díaz-Romero R. Niveles de infección de *Streptococcus mutans* en niños menores de dos años y sus madres en el Instituto Nacional de Perinatología. [Levels of *Streptococcus mutans* infection in children under two years of age and their mothers at the National Institute of Perinatology] *Perinatol Repord Hum*, 20: 27–32, 2006.
20. Ersin NK, Eronat N, Cogulu D, Uzel A, Aksit S. Association of maternal-child characteristics as a factor in early childhood caries and salivary bacterial counts. *J Dent Child (Chic)*, 73: 105–111, 2006.
21. Vargas CM, Ronzio CR. Disparities in early childhood caries. *BMC Oral Health*, 6 (Suppl 1): S3, 2006.
22. Instituto Nacional de Estadística Geografía e Informática. XII Censo General de Población y Vivienda 2000. [National Statistical Institute Geography and Informatics. XII General Census of Population and Housing]. Available at: <http://www.inegi.gob.mx/est/contenidos>.
23. World Health Organization. Oral Health Surveys: Basic Methods. 4th ed. Geneva Switzerland: WHO; 1997.
24. Ismail AI, Brodeur JM, Gagnon P, Payette M, Picard D, Hamalian T, Olivier M, Eastwood BJ. Prevalence of non-cavitated and cavitated carious lesions in a random sample of 7–9-year-old schoolchildren in Montreal, Quebec. *Community Dent Oral Epidemiol*, 20: 250–255, 1992.
25. Thitasomakul S, Thearmontree A, Pivat S, Chankanka O, Pithpornchaiyakul W, Teanpaisan R, Madyusoh S. A longitudinal study of early childhood caries in 9- to 18-month-old Thai infants. *Community Dent Oral Epidemiol*, 34: 429–36, 2006.
26. AAPD: Definition of Early Childhood Caries (ECC) classification, consequences, and preventing strategies. *Pediatr Dent*, 25 (suppl): 31–32, 2004.
27. Segovia-Villanueva A, Estrella-Rodríguez R, Medina-Solís CE, Maupomé G. Dental caries experience and factors among preschoolers in southeastern Mexico: a brief communication. *J Public Health Dent*, 66: 88–91, 2006.
28. Velasco Moncada SI, Araiza Téllez MA, Valenzuela Espinoza E. Asociación de los grupos sanguíneos y caries dental en preescolares. [Association of blood groups and dental caries in preschoolchildren] *Rev Odontol Mex*, 9: 59–64, 2005.
29. Maupomé- Carvantes G, Jaramillo-Lanchero RD, Andrade-Delgado LC, Juárez-Reyes PL, López-Pérez R, Sánchez-Navarro W, Sánchez-Pérez L, Vásquez-Obregón VH. [Fluoride content of table salt in Mexico City]. *Bol Oficina Sanit Panam*, 119: 195–201, 1995.
30. Martínez-Mier EA, Soto-Rojas AE, Buckley CM, Stookey GK, Zero DT, Margineda J. Evaluación del contenido de flúor en sal de mesa fluorada. [Evaluation of the fluoride content of table salt]. *Salud Publica Mex*, 46: 197–198, 2004.
31. Cook SL, Martínez-Mier EA, Dean JA, Weddell JA, Sanders BJ, Eggertsson H, Ofner S, Yoder K. Dental caries experience and association to risk indicators of remote rural populations. *Int J Pediatr Dent*, 18: 275–283, 2008.
32. Newbrun E. *Cariology*. Ed. The Williams & Wilkins Company, Baltimore; 16, 1978.
33. Loyola-Rodríguez JP, Martínez-Martínez RE, Flores-Ferreya BI, Patiño-Marin N, Alpuche-Solis AG, Reyes-Macias JF. Distribution of *Streptococcus mutans* and *Streptococcus sobrinus* in saliva of Mexican preschool caries-free and caries-active children by microbial and molecular (PCR) assays. *J Clin Pediatric Dent*, 32: 121–126, 2008.
34. Beauchamp J, Caulfield PW, Crall JJ, Donly K, Feigal R, Gooch B, Ismail A, Kohn W, Siegal M, Simonsen R. Evidence-based clinical recommendations for the use of pit-and-fissure sealants. A report of the American Dental Association Council on Scientific Affairs. *J Am Dent Assoc*, 139: 257–268, 2008.