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Original Article

Dental Plaque Caries Related Microorganism in Relation to Demographic Factors among a Group of Iraqi Children

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Abstract

Introduction: *Streptococcus mutans* and lactobacilli are most important bacteria in the pathogenesis of dental caries. Cariogenic microflora has been associated to the primary caregiver transmission and sugary diets.

Aim: This study aimed to relate *S. mutans* and lactobacilli in the plaque to demographic factors in Iraqi children.

Materials and methods: The study included 135 children aged 3-10 years. Samples of their dental plaque were taken from the upper second primary molars on the buccal surfaces using a clean toothpick. Each sample was stored in 1 ml of normal saline followed by dispersion for 30 seconds using Vortex mix. Before inoculation, serial dilutions were done. Inoculation was done in the selective media for each microorganism: mitis salivarius-bacitracin agar for *S. mutans* and Rogosa agar for lactobacilli. The bacterial count was estimated by counting CFU and a dissection microscope (×15). A questionnaire on the demographic factors was sent to each child to be filled in by their parents.

Results: *S. mutans* was demonstrated to be greater in the dental plaque of 3-6-year-old children whose mothers are housewives, groups that add sugar to milk, groups without any history of systemic disease, mother's age, and normal-weight children, whereas lactobacilli increased in the plaque of children aged 7–10 years, women, children with fathers who are government employees, mother's occupation, children who were not nursed during sleep, father's age, normal weight, and times of teeth brushing.

Conclusion: *S. mutans* and lactobacilli are clearly associated with different demographic factors. Education of parents and children has an effective impact on controlling their number, reducing dental caries.

Keywords

demographic factors, health, lactobacilli, nutrition, oral plaque, *Streptococcus mutans*

INTRODUCTION

The oral cavity includes several areas that act as niches, such as the tongue, gingival sulcus, periodontal pockets, saliva, surfaces of teeth and cheeks, and soft and hard palates, among others. Each area of the mouth has its distinct characteristics and unique microenvironments that allow oral microbiome to establish and develop. These regions support the oral microbiome which is prevalent with over 700 different species. *Streptococcus mutans* has been documented by epidemiological studies, to be the most common pathogens isolated from the dental plaque of humans.[1]

While studies showed that lactobacilli are more significant in the progression of caries lesions and the onset of low percentage of coronal caries, studies have shown *S. mutans* to be strongly associated with the initiation of dental caries lesion.[2]

The most common types of microbes causing dental caries were found to vary according to race and age of the host, and also vary among individuals of the same race.[3] A study by Marsh showed that the microbiomes found in dental plaque were closely correlated with severe ECC. The study also stated that certain components of this microbiota, particularly *S. mutans* and *S. sobrinus*, are the main etiological factors in the starting and development of caries in oral cavity.[4] Risk factors correlated with starting and development of dental caries are bacterial counts of mutans streptococci and lactobacillus in saliva. *S. mutans* have an important contribution in the onset of caries while lactobacillus has its role in the progression of caries.[5]

The growth of a child and further development are highly affected by nutrition, thereby the type and amount of food consumption and eating habits throughout the child's life have an important impact on the overall health, and on the of the oral cavity health. Imbalanced nutrition has a strong effect on tooth development within the alveolar arch and the following tooth mineralization. This effect could be due to nutritional imbalance.[6]

AIM

This study was designed to assess the level of the dental plaque cariogenic microorganisms of *Streptococcus mutans* and *Lactobacillus spp.* It also aimed to study the relation of the above dental plaque cariogenic microorganisms' level to demographic factors (such as age, gender, nursing type, sleep nursing, adding sugar to milk, tooth brushing, mother's and father's occupation), and in relation to nutritional status of the children.

MATERIALS AND METHODS

The children who participated in this study were 135 in the age range of 3-10 years. To be included in this study, they should have active carious lesion. The children involved were from kindergartens, primary schools and children attending the Department of Pediatric and Preventive Dentistry at the teaching hospital of College of Dentistry, Baghdad, Iraq. Approval was obtained from the scientific committee at the Department of Pedodontics and Preventive Dentistry and from the research ethics committee of the College of Dentistry, University of Baghdad, Iraq (project No. 817323, 30 August 2023). Informed consent was obtained from parents or guardians of all subjects involved in the study.

Samples of dental plaque were taken from the upper second primary molars on the buccal surfaces (sound surface free of carious lesion) by using a clean toothpick (if the second molar was not present then plaque sample was taken from the upper first primary molar). Each sample was stored in 1 ml of normal saline in Eppendorf tube to prevent dryness. Sample dispersion was done for 30 seconds using Vortex mix. Before inoculation, serial dilutions by normal saline (ten-fold) were done in order to obtain clear CFU. Inoculation was then done in the selective media for each microorganism: mitis salivarius-bacitracin agar was used for the *S. mutans*, while Rogosa agar was used for the lactobacilli. Bacterial count was estimated by counting CFU based on the characteristic morphology and by using a dissection microscope (×15).[7]

A questionnaire was sent to each child to be filled in by their parents and returned the next day. Parents of children attending the teaching hospital completed the questionnaire at the same visit. This questionnaire included questions on the demographic factors to be studied.

Statistical analysis

Data description, analysis and presentation were performed using SPSS version 22 (2020, Chicago, Illinois, USA). The tests included descriptive statistics, ANOVA and Games-Howell. Significance was at $p<0.05$.

RESULTS

The distribution of the total sample by the studied demographic factors is shown in **Table 1.**

Comparing the bacterial counts of *S. mutans* in the dental plaque according to the studied demographic factors, we found that *S. mutans* count was statistically significant according to the age groups (in 3-6-year-olds it was significantly higher than in the 7-10-year-olds), mother's occupation (children whose mothers were housewives had a significantly higher count than those with mothers government employees), adding sugar (groups with adding sugars was significantly higher than groups which did not add sugar), medical history (group without any history of systemic disease had significantly higher *S. mutans* count than the group with a positive systemic disease).

In addition, there were significant differences when comparing the *S. mutans* count according to mother's age, and nutritional status **(Table 2)**. Then with the multiple pairwise comparisons as post-hoc test, we found that concerning the mother's age, children whose mothers were 21-30 years old had a significantly higher count than those aged 15-20 years. Regarding the nutritional status, the normal weight children had a significantly higher *S. mutans* count than the overweight children **(Table 3)**.

About the lactobacilli counts, the comparison according to the age group revealed that the 7-10-year group had a significantly higher count than the 3-6-year group, female participant had a significantly higher count than male participants, children with fathers government employees had a significantly higher count than those children with fathers with free jobs, the same for the mother's occupation, and children who was not nursed during sleep had a significantly higher count than the children who were nursed during sleep. Furthermore, there were significant differenc-

Table 1. Descriptive statistics of the total sample according to the studied demographic factors

Variables	Categories	N	%
Age	3-6 years	80	59.26
	7-10 years	55	40.74
Sex	Male	69	51.11
	Female	66	48.89
Father's age	15–20 years	17	12.59
	21–30 years	74	54.81
	31–40 years	33	24.44
	41–50 years	11	8.15
Mother's age	15–20 years	54	40.00
	21–30 years	55	40.74
	31–40 years	26	19.26
Father's occupation	Government employee	66	48.89
	Free jobs	69	51.11
Mother's occupation	Government employee	109	80.74
	Housewife	26	19.26
Medical history	No history	123	91.11
	Positive history	12	8.89
Adding sugar	Yes	39	28.89
	No	96	71.11
Nursing type	Breast feeding	93	68.89
	Bottle feeding	15	11.11
	Mixed	27	20.00
Sleep nursing	Yes	86	63.70
	No	49	36.30
Nutrition	Normal weight	78	57.78
	Overweight	31	22.96
	Obese	26	19.26
Tooth brushing	No brushing	14	10.37
	Once	56	41.48
	Twice	65	48.15

es when comparing the lactobacilli count according to the father's age, the nutritional status, and the times of teeth brushing **(Table 4)**.

Multiple pairwise comparisons revealed that children whose fathers were 15-20 years old had higher lactobacilli count than children with fathers aged 21-30 years, the normal-weight children had a significantly higher lactobacilli count than obese children, and the children who brushed their teeth once or twice had a higher lactobacilli count than the children who did not brush their teeth **(Table 5)**.

DISCUSSION

One of the most complex microbiomes in the body is the oral cavity. Oral bacteria are one of the major factors that have a major role in the dental caries occurrence and further development. However, of the numerous types of oral microorganisms only about 50% have been cultured. Dietary carbohydrates are fermented in the oral cavity by bacteria resulting in acid production. The demineralization of tooth tissues is caused by the resultant acid that leads to caries. During the caries process, a change in equilibrium of microbiota takes place, in which bacteria having acid-production ability and acid-resisting ability could initiate the caries occurrence.[8]

S. mutans has been considered to be highly associated with ECC. It is thought to have a significant role in caries development because of their strong acid-producing and acid-resisting abilities. However, *S. mutans* in patients with caries was found at a low level or absent, while in the caries-free group, it was not found. This suggests that other bacterial species that are closely associated with *S. mutans* may have a major role in the caries development. Bacteria other than *S. mutans* are *Lactobacillus spp* that were considered to be related with dental caries.[9]

A previous study did not find a correlation between the level of streptococcus in saliva and plaque with DMFS and DMFS of upper and lower teeth. Therefore, *S. mutans*, although considered the primary etiological factor of dental caries, had levels that were not found to be associated with high caries activity.[10]

The level of *S. mutans* in saliva and plaque has been studied and no difference was found. It was explained that saliva causes detachment of bacteria from plaque deposits on tooth surfaces, releasing bacteria in saliva in levels that can be measured. Therefore, the levels of *S. mutans* in saliva and plaque may be similar. However, occlusal plaque had higher *S. mutans* counts than did saliva samples, suggesting that plaque may be more reliable than saliva for detecting the high levels of *S. mutans*. The reason for this difference may be that the oral plaque is the main habitat for *S. mutans* and thus a better source of this bacteria. In addition, as *S. mutans* detaches from plaque into saliva during regular salivary clearance, saliva may be a comparable proxy. Overall, regardless of dentition stage, occlusal plaque and saliva can be considered as efficient sources for *S. mutans* detection and quantification.[11]

Parent education, knowledge and occupation have been seen to be related to dental caries. When comparing the percentages of dental caries in children whose parents' education level was less than a bachelor's degree to children whose parents had a bachelor's degree, the non-bachelor group had a greater percentage.[12]

Increased *S. mutans* count associated with housewife mothers found in this study was probably related to the knowledge of the mother. Most mothers tended to taste the food before feeding their children that increases the chance of passing the bacteria to the child's oral environment. This

Table 2. Bacterial counts of *S. mutans* in the dental plaque according to the studied demographic factors (ANOVA)

Variables		Mean	SE	Statistics	*P* value
Age	3–6 years	1.075	0.200	2.871	0.005*
	7–10 years	0.329	0.117		
Sex	Male	0.712	0.193	0.460	0.646
	Female	0.833	0.177		
Mother's age	15–20 years	0.206	0.117	6.911	0.001*
	21–30 years	1.210	0.240		
	31–40 years	1.016	0.329		
Father's age	15–20 years	0.588	0.359	2.077	0.124
	21–30 years	0.576	0.153		
	31–40 years	0.831	0.274		
	41–50 years	2.182	0.630		
Father's occupation	Government employee	0.811	0.202	0.295	0.769
	Free jobs	0.733	0.170		
Mother's occupation	Government employee	0.573	0.127	2.509	0.018*
	Housewife	1.602	0.390		
Adding sugar	Yes	1.226	0.297	1.956	0.056 (0.025) *
	No	0.586	0.136	2.245	
Medical history	No history	0.827	0.143	3.970	0.000*
	Positive history	0.200	0.067		
Nursing type	Breast feeding	0.607	0.140	1.805	0.169
	Bottle feeding	1.043	0.371		
	Mixed	1.186	0.389		
Nutrition	Normal weight	1.058	0.206	4.212	0.017*
	Overweight	0.154	0.034		
	Obese	0.647	0.234		
Sleep nursing	Yes	0.896	0.175	1.339	0.183
	No	0.552	0.187		
Teeth brushing	No brushing	0.800	0.422	0.591	0.555
	Once	0.605	0.182		
	Twice	0.907	0.204		

* significant at $p<0.05$

Table 3. Multiple pairwise comparison of the *S. mutans* counts according to maternal age and nutritional status using Games-Howell

Dependent variable: *S. mutans* in dental plaque				
Variables	**Comparison**		**MD**	***P* value**
Mother's age	15–20 years	21–30 years	−1.005	0.001*
		31–40 years	−0.811	0.067
	21–30 years	31–40 years	0.194	0.883
Nutrition	Normal weight	Overweight	0.904	0.000*
		Obese	0.411	0.392
	Overweight	Obese	−0.493	0.113

* significant at $p<0.05$

Table 4. Bacterial counts of lactobacilli in the dental plaque according to the studied demographic factors (ANOVA).

Variables		Plaque *lactobacilli*		F	*P* value
		Mean	SE		
Age	3–6 years	0.824	0.156	2.178	0.013*
	7–10 years	0.358	0.147		
Sex	Male	0.303	0.106	3.102	0.002*
	Female	0.980	0.191		
Mother's age	15–20 years	0.586	0.180	0.103	0.902
	21–30 years	0.637	0.170		
	31–40 years	0.727	0.262		
Father's age	15–20 years	1.800	0.454	5.366	0.005*
	21–30 years	0.285	0.110		
	31–40 years	0.476	0.169		
	41–50 years	1.655	0.504		
Father's occupation	Government employee	1.021	0.187	3.510	0.001*
	Free jobs	0.264	0.108		
Mother's occupation	Government employee	0.771	0.135	5.222	0.000*
	Housewife	0.062	0.018		
Adding sugar	Yes	0.522	0.202	0.650	0.518
	No	0.680	0.134		
Medical history	No history	0.652	0.119	0.506	0.614
	Positive history	0.453	0.303		
Nursing type	Breast feeding	0.689	0.138	0.333	0.718
	Bottle feeding	0.413	0.223		
	Mixed	0.569	0.267		
Nutrition	Normal weight	0.903	0.161	5.074	0.008*
	Overweight	0.465	0.236		
	Obese	0.031	0.013		
Sleep nursing	Yes	0.420	0.113	2.334	0.022*
	No	1.009	0.225		
Teeth brushing	No brushing	0.003	0.002	16.479	0.000*
	Once	0.866	0.202		
	Twice	0.570	0.147		

* significant at $p<0.05$

Table 5. Multiple pairwise comparison of the lactobacilli counts according to father's age, nutritional status, and teeth brushing using Games-Howell.

Variables	Comparison		MD	*P* value
Father's age	15–20 years	21–30 years	1.515	0.021*
		31–40 years	1.324	0.056
		41–50 years	0.145	0.996
	21–30 years	31–40 years	−0.191	0.781
		41–50 years	−1.369	0.090
	31–40 years	41–50 years	−1.179	0.172
Nutrition	Normal weight	Overweight	0.438	0.283
		Obese	0.872	0.000*
	Over-weight	Obese	0.434	0.175
Teeth brushing	No brushing	Once	−0.863	0.000*
		Twice	−0.567	0.001*
	Once	Twice	0.296	0.464

* significant at $p<0.05$

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may in turn be related to how many children this mother has. Mothers with multiple children demonstrated a more improved understanding of the relation between the mother's oral health during pregnancy and the oral health of her child before and after birth. Differences have been demonstrated between the practical knowledge of mothers having multiple children and those of only one child in the effects of parenting on the oral hygiene of infants.[13] Previous research has shown that children living in poverty visited the dentist less frequently, therefore, showed higher prevalence of dental caries. The parents of younger age with higher level of educational attainment, better occupational status that provide higher income, urban residence all exhibited a strong correlation with dental caries, and favorable influence on dental hygiene practices of the children. Dental care for the child is provided when awareness about their dental health is recognized among parents of young ages, have a higher level of educational achievement and an improved occupational status.[14] In a study by Abonayla and Al-waheb, high caries prevalence was linked to aspects related to the socioeconomic situation and the way of life of the families. Their study brought to light the necessity of children for preventive measures and dental health education and enhancement of dental awareness and attitude towards good oral hygiene.[15]

In this study, the count of *S. mutans* and *Lactobacillus spp*. was related to age. *S. mutans* decreased with age while the count of lactobacillus increased. This result disagreed with Sharma et al. whose results showed the children with mutans *S. mutans* colonization were of high percentage and this colonization increased with age and with the increase of the number of teeth (colony-forming units).[16]

Another study stated that *S. mutans* did not need the presence of teeth to be found in the oral cavity. Zhou et al showed that *S. mutans* could be found in predentate newborns at 8 months of age. Their result suggested that *S. mutans* can invade the oral cavity even before tooth emergence. [17] The decrease in *S. mutans* count with increasing age found in this study could be due to increasing oral hygiene practices as the child becomes gradually more aware of oral health. Lactobacilli were found to be more abundant in older children and this result was consistent with that reported by Lee et al. who found the abundance of lactobacilli in the plaque of children 6-12 years old was greater than in those below six years of age. This may be due to the increase in the number of teeth and permanent teeth since *Lactobacillus spp.* was the main pathogen for caries development.[18]

Lactobacilli appeared in girls higher than in boys. This may be due to the fact that females generally develop more quickly than males, with the former having earlier tooth eruption patterns resulting in the tooth being exposed to cariogenic bacteria earlier the males.[19]

S. mutans were found to be higher in children who were healthy than those with a medical history. This came in agreement with Sonbol et al. who found that children with severe hemophilia were significantly more likely to be caries free compared with the healthy subjects. They found the mean number of colonies forming units of *S. mutans* in the healthy group to be significantly greater than in the hemophilic group.[20] While it disagreed with Al Dhaher et al. in which the hemophilic group had a higher count of *S. mutans* and oral lactobacilli in comparison to healthy subject group.[21] In our study, no severe medical condition was reported, but the decrease in the bacterium may be due to the intake of antibiotics during the previous few days. Another study by Yahya et al. showed that stress may cause immunosuppression, which might make those children more susceptible to infection with *S. mutans* or other types of bacteria. Their study also showed that salivary cortisol level had a positive significant association with the viable count of *S. mutans*.[22]

Frequency of consuming added sugar was found to be related to dental caries and a range of child demographic and lifestyle factors. Children who more often consume foods and drinks with added sugar, especially beverages and juices, are at a higher risk of developing dental caries.[23] This came in line with the result in this study in which adding sugar to the child's milk increased the count of *S. mutans*. The two factors that were mostly related to caries formation were drinking sugar-sweetened drinks and not cleaning teeth on a regular basis. The decreased prevalence of dental caries in those who brush their teeth may be due to the bristles of a toothbrush that mechanically reach and clean the inaccessible areas of the oral cavity that are not reached by finger or other materials. Cleaning teeth will remove the food debris away from the oral cavity. Therefore, *S. mutans* will not be capable of getting enough nutrients and time for growth so no acid production will be present which causes dental caries development.[24] The least number of lactobacilli found in the no-brushing group **(Table 1)** may be attributed to the number of children in this group which were 14 while the other groups (brushing once, brushing twice) were 56 and 65, respectively. A further study of the effect of brushing teeth should be done taking into consideration the equal number of participants that do or do not brush their teeth.

Normal-weight children showed the highest counts in both *S. mutans* and lactobacilli. This finding disagreed with several studies. In contrast to those studies and in line with this study, is a study conducted by Raju. *Veillonella*, *Prevotella*, *Selenomonas*, and *Streptococcus*, considered as core bacteria, were found to be decreased in overweight and obese children. Their study differed from this study in that the samples were from saliva not plaque and the age range of the participants was 11–14 years.[25] An Iraqi study on the effect of nutritional status on dental caries stated there was no significant difference between the three groups (normal, overweight and obese) and their nutritional status.[26]

CONCLUSION

It can be concluded that both *S. mutans* and *Lactobacilli spp.* are clearly related to various demographic factors. Pos-

itive changes in oral health may be brought about by education of the parents and children, intervention and prevention. Improving oral health and controlling the growth of these bacterium in an effort to decrease the dental caries among children can be aided by an effort among parents, guardians, children, and dental professionals.

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Conflicts of Interest

The authors have declared that no competing interest exist.

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Author Contributions

All authors contributed equally to this research.

Ethical approval

Approval was obtained from the scientific committee at the Department of Pedodontics and Preventive Dentistry and from the research Ethics Committee of the College of Dentistry, University of Baghdad, Iraq (project no. 817323, Date: 30 August 2023).

Informed consent statement

Informed consent was obtained from parents/guardians of all subjects involved in the study.

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Микроорганизмы в зубном налёте, вызывающие кариес в связи с демографическими факторами в группе иракских детей

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Резюме

Введение: *Streptococcus mutans* и лактобациллы являются наиболее важными бактериями в патогенезе кариеса зубов. Кариесогенная микрофлора связана с людьми, обеспечивающими режим питания и с наличием сладких продуктов в рационе питания.

Цель: Целью данного исследования было связать *S. mutans* и лактобациллы в зубном налёте с демографическими факторами у иракских детей.

Материалы и методы: В исследовании приняли участие 135 детей в возрасте от 3 до 10 лет. Образцы их зубного налёта были взяты с верхних вторых молочных моляров на щёчных поверхностях с помощью чистой зубочистки. Каждый образец хранился в 1 ml физиологического раствора с последующим диспергированием в течение 30 секунд с использованием смеси Vortex. Перед инокуляцией были сделаны серийные разведения. Инокуляция была сделана в селективной среде для каждого микроорганизма: агар Mitis salivarius-bacitracin для *S. mutans* и агар Rogosa для лактобацилл. Количество бактерий оценивалось путём подсчёта CFU и с использованием диссекционнго микроскопа (×15). Каждому ребёнку была отправлена анкета по демографическим факторам для заполнения родителями.

Результаты: Было показано, что *S. mutans* содержится в больших уровнях в зубном налёте у детей в возрасте 3–6 лет, чьи матери являются домохозяйками, в группах, которые добавляют сахар в молоко, в группах без наличия системных заболеваний и при демографическим факторе – возраст матери и дети с нормальным весом, тогда как уровень лактобацилл был выше в зубном налёте у детей в возрасте 7–10 лет, у детей, чьи отцы являются государственными служащими, у детей, которых не кормили грудью во время сна и при таких демографических факторах, как род занятий матери, возраст отца, нормальный вес и продолжительность чистки зубов.

Вывод: *S. mutans* и лактобациллы явно связаны с различными демографическими факторами. Обучение родителей и детей оказывает эффективное влияние как на контроль количества кариесов, так и снжение прироста кариесов.

Ключевые слова

демографические факторы, здоровье, лактобациллы, питание, зубной налёт, *Streptococcus mutans*