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Salivary Caries-Related Microorganisms and Demographics of a Group of Children

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Abstract

Objective: The purpose of this study was to determine the association between caries related microorganisms in children's saliva, such as *Streptococcus mutans* and *lactobacilli*, and their demographic factors.

Methods: This study involved a sample of 135, both sexes with an age range between 3 and 10 years. Unstimulated saliva was obtained and diluted in normal saline. Saliva was then placed in selective media. Salivaris agar was used for *mutans streptococci* while Rogosa agar for *lactobacilli*. After incubation, *Streptococcus mutans* counting of CFU (colony forming units) with morphology characterization and numbers of CFU per milliliter of saliva for *lactobacilli*. Demographic factors information was collected using a questionnaire.

Results: Both studied salivary microorganisms increased with age. *Streptococcus mutans* was higher in boys, and associated with breastfeeding, overweight, and not brushing their teeth. L*actobacilli* were higher in girls, and associated with mothers who were government employee, mother occupation, children who during sleep were not nursed, and with normal weight.

Conclusion: The present study found an association between salivary *Streptococcus mutans* and *lactobacilli* and various demographic variables in a group of children.

Keywords: Saliva, *Streptococcus mutans*, *Lactobacilli*, Health, Demographics.

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Introduction

The surfaces of the oral cavity are covered by salivary fluids, which is produced via the salivary glands; this fluid has definitive essential roles for maintaining oral healthy (1). Many bacteria are present in saliva [2]. In fact, saliva secreted into the oral cavity from salivary glands is sterile [3], but when washing with saliva, bacteria shed to saliva from different surfaces in the oral cavity including oral mucosa, gingival crevices, and dental plaque, but the main site of origins are throat, tongue and the tonsils [4]. The microbiota of saliva contains many species of bacteria that are temporally stable and have been shown to be individualized, playing a crucial role in disease as dental caries and health of oral cavity [5]. Public policies on oral health consistently address several factors associated with dental caries like oral hygiene rule, dietary habits, genetics, oral health knowledge, demographics and sociocultural environment. Many studies have revealed an influence of diet and lifestyle on microbiota of saliva [6,7]. It is a fact that dental developmental stages affected the microbiome of saliva. Key factors include teeth eruption, age, oral hygiene practices and dietary changes [8].

Dental caries is a chronic disease which is widespread and affect people of different ages and demographics [9]. It is caused by bacterial acid result from fermentation of free sugars and poor oral hygiene, leading to localized damage to the hard tooth tissue, associated with the structure of deciduous and permanent teeth [10,11]. Most types of cariogenic bacteria were found to differ according to host, age and race. Bacteria vary among individuals of the same race. Investigations have employed

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saliva to determine the occurrence of caries by exploring levels of the bacteria. as many cariogenic bacteria are present in saliva. *Mutans Streptococcus* (MS) is categorized as primary contributors to dental caries. Another bacteria strain set linked to the advancing of dental caries is *lactobacillus* (LB). Salivary lactobacilli may have an indirect role with advancement of caries. Elevated concentration of *S. mutans* and *Lactobacillus* in saliva are significantly corelated to the early occurrence of dental caries [12-16]. Saliva is one of the most studied components of caries [15], and because of positive correlation between concentration of salivary SM and LB and caries, these bacteria may be potential indicators for dental caries. A significant increase of salivary SM and LB concentrations was found in a group of adolescents with nontreated caries [16]. This study was intended to investigate the levels of *Streptococcus mutans* and *Lactobacillus* species in saliva and their relationship with children's demographic profiles and nutritional status.

Material and Methods

This research is structured as descriptive observational study with cross-sectional design. It includes children of both sexes and ages 3 to 10 years [17] being treated at the teaching hospital's Pedodontic and Preventive Dentistry unit clinics, University of Baghdad's College of Dentistry. This study enrolled children with active carious lesions. Official approval of study protocol was garneted by both Department of Pedodontics and Preventive Dentistry's Scientific Committee and the College of Dentistry's Institutional Research Ethics Committee at the University of Baghdad in Iraq (reference Number.:816, project Number. 816323, Date;30-8-2023) (Chairman, Prof. Dr. Salwan Y. Bede). The parents of all children included in this study were asked to sign an informed consent document.

Total sample of children participating in this study was 135. Each child received a questionnaire to be filled by their parents. Demographic variables included in this questionnaire were date of birth, age, sex, nursing pattern, nursing at sleep, added sweet to milk, tooth brushing, and occupation of mother and father.

Following standard guidelines of Fejerskov and Thylstrup, in the morning between 10 to11 A.M. the samples of unstimulated saliva were collected [19]. Prior to this procedure, children rinsed their mouths with water and asked to spit the first mouthful of saliva into a small, pre-labeled plastic tube. One hour before collecting saliva, the child was asked not to eat or drink (except for water). A pre–sample period of 1 minute was followed. Then, unstimulated saliva was collected for 10 to 15 minutes. Saliva was collected with the child sitting and relaxed on a common chair. Any samples containing blood were discarded. After completing saliva collection, normal saline was used for dilution of the sample in the laboratory. A micropipette was used for application of saliva on selective media surfaces - mitis salivaris agar targeting *Mutans streptococci* and Rogosa agar for *Lactobacilli.* Then plates anaerobically were incubated at 37°C for 48 hours. For *Streptococcus mutans*, counting of Colony Forming Units (CFU) and morphology features were used, while numbers of CFU per ml of saliva were used for lactobacilli [20].

G power 3.1.9.7 (Program design by Franz-Faul at the University of Kiel, Germany) was used to calculate sample size of this study. Assuming 80% power, alpha error of 0.05, effect size of 0.25 (medium) sample size of this study was 111. When adding 10% as an error rate, 122 subjects were suggested. Therefore, our sample of 135 was enough to detect differences in our study [21].

Daily entrance of data from the children's case sheets was done into Microsoft office Excel 2013, and the Statistical Package for Social Science version 22 (SPSS) (Chicago, Illinois, USA) was implemented for statistical tests.

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We determined frequency, percentages, means and standard errors (SE), unpaired T test, one way analysis of variance with Games-Howell and Pearson correlations. Statistically significant level was accepted at p-value of less than 0.05.

Results

The overall sample were allocated based on the demographic variables as shown in Table 1. Comparison of *Streptococcus mutans* count in the saliva of children with their demographics, data revealed that the bacterial count is significantly increased with increased age. Additionally, the boys had significantly higher count than girls. Furthermore, count of salivary *Streptococcus mutans* showed a significant difference for type of nursing, status of nutrition and brushing time of teeth (Table 2).

The count of bacterial *Streptococcus mutans* in saliva significantly increased in children who did not brush their teeth compared to those who brushed one time (Table 3). The count of *lactobacilli* in the saliva revealed that girls had significantly higher counts compared to boys, and a significant higher bacterial count were noted in children of government employee working mothers than in children of housewives (Table 4). Multiple comparisons for children's nutritional status revealed that the differences were significant when comparing the obese group with the two other groups (Table 5).

Discussion

Dental caries is multifactorial in nature, so salivary features, oral hygiene, nutritional status, and demographic factors should be registered to indicate their roles as risk factors for dental caries [22]. Saliva provides a complex mixture of microorganisms and collection of saliva is noninvasive and easy [5]. *Streptococcus mutans* detach to saliva from different oral surfaces and is considered as major cariogenic bacteria. L*actobacilli* play a role in tooth decay development because it can produce extracellular polysaccharides and lactic acid [23]. Several studies have investigated the salivary bacterial levels of *Streptococcus mutans* and risk of dental caries [22,24]. For optimal health from childhood to adulthood, good oral hygiene practices throughout life are suggested [25,26]. Changes in the environment and lifestyle shifts such as high access to consumption of processed foods with an increased content of carbohydrates and urbanization have contributed to obesity and dental caries among children [27].

The finding of this study showed an increase of salivary *Streptococcus mutans* and *lactobacilli* counts among children with increased age, in agreement with previous data [28,29].

In general, girls grow faster than boys, which results in earlier teeth eruption and more exposure to cariogenic bacteria [30]. Many studies observed variations in the salivary microbiota between males and females, which might suggest that microorganisms have limited influence on determination of dental caries [31,32].

Parents are responsible for providing a suitable environment for a healthy lifestyle and instilling good habits of oral hygiene [33]. In our study, children from mothers that were government employees had significantly higher counts of *lactobacilli* than children whom mothers were housewives, which agrees with previous work [34]. This could be since housewives put more effort in maintaining children's oral health because they spend more time with them.

Streptococcus mutans counts were higher in breastfeeding than other feeding patterns in our study. This is possibly because mothers

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include foods and drinks other than breastmilk feeding, which increases susceptibility of the children to cariogenic microorganisms' early childhood caries [35,36]. Also, our study showed that overweight children significantly have highest *Streptococcus mutans* counts. The same results were found in other studies [37-39], which linked an unhealthy body mass index or obesity with increased dental caries. Khalaf et al. and Raju et al. found inverse results, as their results showed that *Streptococcus mutans* counts in children with normal weight were higher than other BMI weights [40,41]. Children who did not brush their teeth had significantly higher *Streptococcus mutans* counts in their saliva in our study findings, which agrees with previous work [40]. Mechanical action of toothbrush bristles and active anticaries effects of toothpaste may provide this benefit of tooth brushing in decreasing the prevalence of dental caries. Brushing leads to not enough nutrients and time for growth of *Streptococcus mutans* and this led to a decrease in acid production [42].

This study has some potential limitations. The sample may not represent the broader population. Also, because this study is a cross-sectional in design, it could not give the causal relation between salivary caries related microorganisms and development of caries, depending on different demographics. In addition, our study sample collection from a specific geographic and socioeconomic group may not apply to other populations or communities.

Conclusion

Microbial profile of both salivary *Streptococcus mutans* and *lactobacilli* correlate with demographic factors of children. Further investigations using longitudinal designs are needed to search for causative relations and the role of demographics.

Ethical Approval

This study was received ethical approval from the Research Ethics Committee of the College of Dentistry \ University of Baghdad Ref. number: 816, Date 30-8-2023.

Conflicts of interest

The authors advertise no competing interest.

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Table 1. Descriptive statistics of the complete sample depending on the studied demographic variables.

Demographic Variables	Divisions	No.	%
Child age	3 to 6 years	80	59.26
	7 to10 years	55	40.74
Child sex	Males	69	51.11
	Females	66	48.89
Father occupation	Government employee	66	48.89
	Free jobs	69	51.11
Mother occupation	Government employee	109	80.74
	Housewife	26	19.26
Adding sugar	Yes	39	28.89
	No	96	71.11
Type of nursing	Breast milk feeding	93	68.89
	Bottle milk feeding	15	11.11
	Mixed	27	20.00
Sleep nursing	Yes	86	63.70
	No	49	36.30
Nutritional status	Normal weight percentile	78	57.78
	Over-weight percentile	31	22.96
	Obese percentile	26	19.26
Tooth brushing	No brushing	14	10.37
	One time	56	41.48
	Two time	65	48.15

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Table 2. The counts of *S. mutans* level in the saliva based on the demographic variables.

Demographic Variables		Mean	SE	Test	P value
Child age	3-6 years	.220	.031	3.308	**0.001**
	7-10 years	.462	.066		
Child sex	Males	.419	.054	3.106	**0.001**
	Females	.214	.038		
	21-30 years	.239	.041		
	31-40 years	.604	.083		
	41-50 years	.345	.016		
Father occupation	Government employee	.252	.042	1.931	0.056
	Free jobs	.382	.053		
Mother occupation	Government employee	.321	.041	0.254	0.801
	Housewife	.306	.044		
Adding sugar	Yes	.248	.066	1.282	0.204
	No	.347	.040		
	Positive history	.671	.199		
Type of nursing	Breast milk feeding	.386	.044	12.992	**0.000**
	Bottle milk feeding	.027	.018		
	Mixed	.247	.061		
Nutritional status	Normal weight percentile	.357	.039	6.416	**0.003**
	Over-weight percentile	.424	.102		
	Obese percentile	.076	.024		
Sleep nursing	Yes	.348	.046	1.237	0.219
	No	.266	.047		
Teeth brushing	No brushing	.711	.154	6.744	**0.004**
	One time	.205	.047		
	Two time	.332	.041		

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Table 3. Games-Howell test for multiple comparing of S*. mutans* in saliva.

Demographic Variables	Comparisons		MD	P value
Type of nursing	Breast milk feeding	Bottle feeding	0.360	**0.000**
		Mixed feeding	0.139	0.165
	Bottle milk feeding	Mixed feeding	-0.220	**0.005**
Nutrition status	Normal weight percentile	Over-weight	-0.067	0.813
		Obese percentile	0.281	**0.000**
	Over-weight percentile	Obese percentile	0.348	**0.006**
Tooth brushing	No brushing	One time	0.507	**0.017**
		Two time	0.380	0.076
	One time	Two time	-0.127	0.111

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Table 4. The counts of *lactobacilli* level in the saliva based on the demographic variables.

Demographic Variables		Saliva *lactobacilli*		F	P value
		Mean	SE		
Child Age	3 to 6 years	.221	.036	1.771	0.081
	7 to 10 years	.372	.077		
Child Sex	Boys	.128	.033	4.320	**0.000**
	Girls	.444	.065		
Father occupation	Government employee	.281	.046	0.042	0.967
	Free jobs	.284	.061		
Mother occupation	Government employee	.316	.047	3.086	**0.003**
	Housewife	.143	.031		
Adding sugar	Yes	.302	.054	0.367	0.714
	No	.275	.050		
Type of nursing	Breast milk feeding	.275	.052	0.449	0.639
	Bottle milk feeding	.383	.062		
	Mixed feeding	.253	.066		
Nutritional status	Normal weight percentile	.359	.061	9.524	**0.000**
	Over-weight percentile	.285	.053		
	Obese percentile	.052	.015		
Sleep nursing	Yes	.215	.029	2.373	**0.019**
	No	.402	.091		
Teeth brushing	No brushing	.154	.070	0.954	0.388
	One time	.263	.052		
	Two time	.327	.065		

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Table 5. Games-Howell test for multiple Comparing of *lactobacilli* in saliva.

Variables	Comparisons		MD	p value
Nutritional status	Normal weight percentile	Over-weight percentile	0.074	0.634
		Obese percentile	0.307	**0.000**
	Over-weight percentile	Obese percentile	0.234	**0.000**