Correlation between Caries Related Microorganisms in the Dental Plaque and Saliva with Dental Caries Level in the Upper and Lower Jaws in 5-9 Years Old Children in Baghdad City

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ABSTRACT

Background: The oral cavity is considered as a complex ecological niche, its complex microbial community is reflected to it. *Streptococcus mutans* has been implicated as one of the major etiological factor of dental caries. Tooth surfaces colonized with *Streptococcus mutans* are at a higher risk for developing caries, while *lactobacilli* are considered as the secondary invaders, not initiators of the carious lesion. The main purpose of this study was to correlate the dental caries (for primary and permanent teeth) in the upper jaw with the *streptococcus mutans* and *lactobacilli* count in the dental plaque and saliva, also to correlate the dental caries (for primary and permanent teeth) in the lower jaw with the *streptococcus mutans* and *lactobacilli* count in the saliva.

Materials and methods: Forty seven children aged 5-9 years old were selected for this study. Dental caries recording was carried out by the dmfs index (decayed, missed, filled surfaces for primary teeth) to inspect the primary teeth and DMFS index (decayed, missed, filled surfaces for permanent teeth) to inspect the permanent teeth, by using the dental mirror and explorer. Collection of salivary samples was performed in the morning between (10-11) a.m. at least one hour after breakfast, then normal saline was added to have tenfold dilutions, for the purpose of full colony counting of the caries related microorganisms (*streptococcus mutans* and *lactobacilli*), then inoculation was done in the special selective media (for the *streptococcus mutans* is Mitis-Salivarius-Bacitracin agar, and for the *lactobacilli* is Rogosa agar). Counting of the colonies of the bacteria were estimated by the aid of dissection microscope

Results: The highest level of dmfs means was found in primary upper teeth, it was 17.6383 ± 10.10 while for the permanent teeth the mean of DS and DMFS was highest in the lower teeth, it was 0.7391 ± 1. Pearson correlation was used to show the correlation between the ds and dmfs of upper and lower primary teeth with the level of *streptococcus mutans* in saliva (sm. Sal) and lactobacillus in saliva ,there was a significant correlation between the ds and dmfs for upper primary teeth at level 0.01 (2-tailed), and there was negative correlation between dsl and level of *streptococcus mutans* in saliva (sm. Sal) also there was negative correlation between dmfs for upper and lower primary teeth with level of *streptococcus mutans* in saliva, also the correlation between *lactobacillus* level in plaque with *streptococcus* level in plaque was negative, while for upper permanent teeth the correlation was negative with both type of bacteria level in plaque with the DSU and DMFSU

Conclusion: The caries activity was more prominent in upper teeth than lower teeth, levels of streptococcus *mutans* were not associated with high caries activity, which emphasizes and consistent with the fact that the dental caries is a multifactorial disease, related to many factors.

Key words: Dental caries, Dental plaque, Saliva, streptococcus *mutans*, *lactobacilli*. (J Bagh Coll Dentistry 2016; 28(3):132-136).

INTRODUCTION

The oral cavity considered as a complex ecological niche, its complex microbial community is reflected to it. The human microorganisms are widely differ in types, as a result of new technology; studies estimated about 10000 types of microorganisms had been seen in the human dental plaque of 98 healthy adults [(1)], In return, there were lower levels of human oral microorganisms estimated by traditional methods (700 phylotypes for the oral microorganisms) [(2)].

Every site in the oral cavity, such as: mucosal sites, anaerobic pockets, and the hard dental surfaces; has its unique communities if microorganisms [(3,4)].

Physical and chemical fluctuation will dramatically change the ecological system of the oral cavity by the oral hygiene measures, drinks and food ingestion, in addition; lower variation in the oral microorganisms among individuals as compared with skin and gastrointestinal microorganisms (a relatively stable oral microbial community) [(5)].

As a general fact "A shift in microbial composition is an important step in the progression of oral disease", however; this fact is emphasized by few studies. The shift in microorganisms of the mouth is closely related to the oral hygiene. *Streptococcus mutans* has been Involved as one of the major etiological factor of dental caries [(6,7)]. Its colonization on the tooth surfaces is closely related to the development of dental caries [(8)].

There is a positive association between streptococcus mutans levels in saliva and high

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caries experience of the populations [9,10]. Individuals with high levels of *S. mutans* also develop more coronal and root caries in temporary and permanent restorations than do individuals in the same population with lower concentration of *S. mutans* [11,12]. There is a direct relation between streptococcus mutans levels in saliva and the number of colonized tooth sites [13] and to their proportion in dental plaque.[14] Lactobacilli are considered secondary invaders rather than initiators of the caries process [15]. They are not found in incipient caries and are found in less quantity than *Streptococcus mutans* [16] The presence of these micro-organisms is also depend on the size of the cavity: the larger the cavity, the more numerous bacteria [17].

This study aimed to:

1. Correlate the dental caries (primary and permanent teeth) in the upper jaw with the *streptococcus mutans* and *lactobacilli* count in the dental plaque and saliva
2. Correlate the dental caries (primary and permanent teeth) in the lower jaw with the *streptococcus mutans* and *lactobacilli* count in the saliva.

MATERIALS AND METHODS

Children selected for this study were 47 aged 5-9 years. They should have active carious lesion. A subsample of 31 children was taken for the bacterial examination, from primary schools and kindergartens, and the in patients of the department of Pediatric and Preventive Dentistry, Baghdad teaching hospital for Dentistry.

Using mouth mirror and sharp explorer , dental caries was recorded following the WHO 1987 criteria for primary and permanent dentition (dmfs, DMFS respectively) [18].

Collection of salivary samples was performed according to Fejerskov and Thylstrup [19] "in the morning between (10-11) a.m. at least one hour after breakfast, the children were asked to rinse out their mouths with water. After that, the first mouthful of saliva was thrown, while one ml unstimulated (resting) whole saliva was collected into small labeled plastic polyethylene tubes according to spitting method for collection.

The following points should be kept in mind:

1. The patient should not eat or drink (except water) one hour before saliva collection.
2. A pre – sampling period of one minute is recommended.
3. A fixed collection time (10-15 min. for unstimulated saliva) should be used.
4. The patient should sit in a relaxed position in an ordinary chair.
5. Samples containing blood should be discarded if chemical analyses of saliva are planned.

After collection of the saliva, dilution was performed with normal saline in the bacteriology laboratory – college of dentistry - Baghdad University. After that saliva was applied on the surface of the selective media by using micropipette (Mitis salivaris agar and Rogosa agar medium are the selective medium for *mutans streptococci* and *lactobacilli* respectively). After incubation of the plates in an anaerobic atmosphere for 48 hours at 37°C, counting of CFU (colony forming units) with morphology characteristic of s. mutans and *lactobacilli* (numbers of CFU per milliliter of saliva) [20].

Dental plaque sample was taken by a clean toothpich from sound buccal surfaces of upper deciduous molars (the second molar; if it was not found then from the first molar). 1 ml. of normal saline in Epindorf tube was used to store the sample in order not to dry. Vortex mix was used to dispersion for 30 seconds. In order to see clear CFU we have to do serial dilutions by normal saline (tenfold) before inoculation in the selective media for each microorganism (for the streptococcus mutans is Mitis-Salivarius-Bacitracin agar, and for the lactobacilli is Rogosa agar). By using dissection microscope 15 X, basing on the characteristic morphology, counting of the CFU was estimated [22]. Statistical analysis was done by using the IBM SPSS version 19 win 64.

RESULTS

The descriptive statistics was demonstrated in table 1 for decayed surfaces (ds) for upper primary teeth (dsu) and for the lower primary teeth (dsl) while the (DSU)represent the decayed surfaces for upper permanent teeth and (DSL)represent the decayed surfaces for lower permanent teeth the highest levels of dmfs means was found in upper primary teeth it was 17.6383 ± 10.10 while for the permanent teeth the mean of DS and DMFS was highest in the lower teeth, it was 0.7391 ± 1 as shown in table 1.

The descriptive statistics for the colony forming units of *streptococcus mutans* and *lactobacilli* are demonstrated in table 2.

Pearson correlation was used to show the correlation between the ds and dmfs of upper and lower primary teeth with the level of *streptococcus mutans* in saliva (sm. Sal) and lactobacillus in saliva too (lb.sal) as shown in table 3 and 4 .There was a significant correlation between the ds and dmfs for upper primary teeth

at level 0.01 (2-tailed), and there was negative correlation between ds.l and level of *streptococcus mutans* in saliva (sm. Sal) also there was negative correlation between dmfs for upper and lower primary teeth with level of *streptococcus mutans* in saliva as shown in table 3 and 4.

In table 5 the Pearson correlation was used for DS for upper permanent teeth (DSU) and DMFS for upper (DMFSU) with the level of both bacteria in saliva. Correlation was significant as the same for primary teeth between DS and DMFS and there was negative correlation between the level of both bacteria with DMFS for upper teeth. While in table 6 the correlation between DSL and DMFSL with both type of bacteria was positive.

In table 7 the correlation between the level of lactobacillus in the dental plaque. The correlation between bacteria in plaque with the ds and dmfs was negative, also the correlation between lacto bacillus level in plaque with streptococcus level in plaque was negative as shown in table 7, while for upper permanent teeth the correlation was negative with both type of bacteria level in plaque with the DSU and DMFSU as shown in table 8.

Table 1: The descriptive statistics for decayed surfaces and decayed missing filling surfaces in upper and lower primary and permanent teeth

	N	Min	Max	Mean	S.D.
ds.u	47	4.00	41.00	15.2979	8.98287
dmfs.u	47	4.00	46.00	17.6383	10.10042
ds.l	47	.00	42.00	10.7447	8.55791
dmfs.l	47	2.00	42.00	13.5106	9.33174
DS.U	23	.00	3.00	0.4348	.78775
DMFS.U	23	.00	3.00	0.4348	.78775
DS.L	23	.00	4.00	0.7391	1.00983
DMFS.L	23	.00	4.00	0.7391	1.00983

Table 2: The descriptive statistics for the colony forming units of *streptococcus mutans* and *lactobacilli*

	N	Min	Max	Mean	S.D.
sm.pl	31	.000	6.000	.79839	1.582728
lb.pl	31	.000	4.400	.69129	1.322076
sm.sal	31	.000	1.500	.34639	.388449
lb.sal	31	.000	2.000	.28277	.445764

Table 3: Pearson correlation for upper primary teeth with streptococcus and lacto bacillus bacteria

		ds.u	dmfs.u	sm.sal	lb.sal
ds.u	**r**	1	.869**	.039	.305
	p		.000	.834	.095
dmfs.u	**r**	.869**	1	-.052-	.136
	p	.000		.783	.467
sm.sal	**r**	.039	-.052-	1	.233
	p	.834	.783		.207
lb.sal	**r**	.305	.136	.233	1
	p	.095	.467	.207	

****Correlation is significant at the 0.01 level.**

Table 4: Pearson correlation for lower primary teeth with streptococcus and lacto bacillus bacteria

		ds.l	dmfs.l	sm.sal	lb.sal
ds.l	**r**	1	.860**	-.117-	.144
	p		.000	.530	.439
dmfs.l	**r**	.860**	1	-.098-	.101
	p	.000		.601	.588
sm.sal	**r**	-.117-	-.098-	1	.233
	p	.530	.601		.207
lb.sal	**r**	.144	.101	.233	1
	p	.439	.588	.207	

**** Correlation is significant at the 0.01 level.**

Table 5: Pearson correlation for upper permanent teeth with streptococcus and lacto bacillus bacteria level in saliva

		DS.U	DMFS.U	sm.sal	lb.sal
DS.U	**r**	1	1.000**	-.159-	-.346-
	p		.000	.542	.174
DMFS.U	**r**	1.000**	1	-.159-	-.346-
	p	.000		.542	.174
sm.sal	**r**	-.159-	-.159-	1	.233
	p	.542	.542		.207
lb.sal	**r**	-.346-	-.346-	.233	1
	p	.174	.174	.207	

****Correlation is significant at the 0.01 level.**

Table 6: Pearson correlation for lower permanent teeth with streptococcus and lacto bacillus bacteria level in saliva

		DS.L	DMFS.L	sm.sal	lb.sal
DS.L	**r**	1	1.000**	.014	.112
	p		.000	.956	.668
DMFS.L	**r**	1.000**	1	.014	.112
	p	.000		.956	.668
sm.sal	**r**	.014	.014	1	.233
	p	.956	.956		.207
lb.sal	**r**	.112	.112	.233	1
	p	.668	.668	.207	

****Correlation is significant at the 0.01 level.**

Table 7: Pearson correlation for upper primary teeth with streptococcus and lacto bacillus bacteria in plaque

		ds.u	dmfs.u	sm.pl	lb.pl
ds.u	**r**	1	.869**	.355	-.155-
	p		.000	.050	.404
dmfs.u	**r**	.869**	1	.331	-.050-
	p	.000		.069	.790
sm.pl	**r**	.355	.331	1	-.018-
	p	.050	.069		.923
lb.pl	**r**	-.155-	-.050-	-.018-	1
	p	.404	.790	.923	

****Correlation is significant at the 0.01 level.**

Table 8: Pearson correlation for upper permanent teeth with streptococcus and lacto bacillus bacteria in plaque

		DS.U	DMFS.U	sm.pl	lb.pl
DS.U	**r**	1	1.000**	-.249-	-.303-
	p		.000	.335	.237
DMFS.U	**r**	1.000**	1	-.249-	-.303-
	p	.000		.335	.237
sm.pl	**r**	-.249-	-.249-	1	-.018-
	p	.335	.335		.923
lb.pl	**r**	-.303-	-.303-	-.018-	1
	p	.237	.237	.923	

****Correlation is significant at the 0.01 level.**

DISCUSSION

Due to the high prevalence in all regions of the world and the greatest impact on the socially marginalized populations, oral disease is a major public health problem. Therefore, the evaluation of caries risk is most important. It is mandatory to improve diet, hygiene, and preventive measures in an exposed population [23]. In this study the caries activity was more prominent in upper teeth than lower teeth and this is due to the fact that the continuous pool of saliva from sublingual glands makes the teeth immune against the carious lesion by the antigens found in the saliva and by the self cleansing action of the tongue [24,25]. The correlation between streptococcus level in saliva and plaque with dmfs and DMFS of upper and lower teeth was negative and this was in accordance to study done for adult individuals which report, levels of *streptococcus mutans* were not associated with high caries activity, "*mutans streptococci* have been typically considered the primary etiological agents of dental caries", although it is still a subject to talk. In addition to its aciduric and acidogenic properties, the extracellular polysaccharide synthesis, biofilm will form from sugar constitutes which is one of the most important key virulence factors of *streptococcus mutans* [26]. There was no difference in correlation between the CFU of both type bacteria in dental plaque and saliva which is in accordance with the result of study done by Mundroff et al who proved that the number of *streptococcus mutans* or lactobacillus in dental plaque does not explain variation in dental caries better than the bacterial CFU in whole saliva [27]. Levels of streptococcus mutans were not associated with high caries activity, which emphasizes and consistent with the fact that the dental caries is a multifactorial disease and related to many factors such as: feeding at night with sweets [28], people's lifestyle and socioeconomic status [29-31], ingestion of fermentable carbohydrates [28,30], sugar intake, presence of plaque [31,32], lack of tooth-brushing with fluoride toothpaste [33,34], lack of strict dietary control and regular oral hygiene measures [34], mother education [29], and so on.

From this study we concluded that there is not one particular bacterial species responsible for caries production. The caries activity was more prominent in upper teeth than lower teeth, levels of streptococcus mutans were not associated with high caries activity, which emphasizes and consistent with the fact that the dental caries is a multifactorial disease, related to many factors.

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