

Correlation of Streptococci mutans, Oral Lactobacilli and Oral Enterococci with dmft Score in Iraqi Children

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Abstracts

Purpose: The objective of this study was to correlate Streptococci mutans, oral lactobacilli and oral enterococci counts with dmft scores in children 4-9 years of age.

Methods: unstimulated saliva were collected in sterile tubes, diluted and plated on three selective media including Mitis-Salivarius Bacitracin agar (MSB), Rogosa SL agar and m Enterococcus agar. Sampling methods were compared by frequency of recovery of streptococci mutans, oral lactobacilli and oral enterococci correlation of microbial counts with dmft scores.

Results: Mean dmft for thirty three children was 6.3. Significant relation was observed between Streptococci mutans and dmft ($p=0.053$) whereas no significant relation were found between oral lactobacilli and oral enterococci with dmft. The three isolated bacteria showed no significant relation with salivary pH.

Conclusions: Higher value for dmft was obtained for random sample of Iraqi children. Streptococci mutans was a strong indicator of caries status than oral lactobacilli and oral enterococci.

Introduction

Dental caries is widely recognized as an infectious disease induced by diet. The main players in the etiology of the disease are cariogenic bacteria, fermentable carbohydrates and a susceptible tooth⁽¹⁾. Among the three factors described above, bacteria have been suggested to have the strongest effect on the prevalence or incidence of dental caries⁽²⁾. It is still a serious public health problem and its control should be a priority, since it may lead to malocclusion of permanent teeth, cause phonetic problems and lower self-esteem.⁽³⁾ The prevalence of caries in the general population is not well-defined, due to different methodologies, which hamper the comparison of results⁽⁴⁾. However, it affects 60-90% of school-aged children in industrialized countries⁽⁵⁾. Different kinds of Gram-positive bacteria are closely related to the formation and progression of dental caries⁽⁶⁾. The main cariogenic microorganisms are the so-called mutans streptococci, especially Streptococcus mutans (MS)⁽⁷⁾. A lot of researches have been done to find out and prove the influence of salivary MS on caries experience in children population⁽⁸⁾. Lactobacilli, which consistently be isolated from established caries lesions in humans but do not show an association with initiation of the disease⁽⁹⁾, has an observed correlation with caries⁽¹⁰⁾. It has been suggested that salivary level of Streptococci mutans and lactobacilli correlate with number of dmft⁽¹¹⁾. Enterococci were the first bacteria shown experimentally to induce caries in gnotobiotic animals⁽¹²⁾. In the oral cavity, E. faecalis is often found in caries and lesions associated with periodontal diseases⁽¹³⁾.

Methods

A random sample of 33 healthy children (25 males & 8 females) with an age range 4-9 years old (Mean age was 6.4 years) was selected at different primary schools and kindergartens in Mosul city, north of Iraq. All children were volunteers, with permission to take part in this study from their parents. Children with systemic diseases, antibiotics taking within last three weeks and topical fluoride application were excluded. Deciduous teeth were examined with the aid of disposable plane mirrors and dental explorers under a good light. Finding was recorded in the term of dmft (decayed, missing and filled teeth) for each child according to the WHO standard⁽¹⁴⁾. Three millimeter of unstimulated whole saliva samples were

obtained from subjects. Samples were immediately put on ice and transported to the laboratory. One milliliter of saliva was used to record pH by using papery pH meter. The remainder of saliva samples were shaken for 30 s and serially diluted (1:10, 1:100 till 1:1000000) in isotonic saline solution. 0.1 ml of each dilution of saliva specimen was homogeneously spread on the surface of the selective media.

The samples were then inoculated in duplicate on three types of selective media used in this study; Mitis Salivarius agar (Himedia, India) supplemented with 0.25 U Bacitracin (Taro pharmaceuticals, USA) and 1% tellurite solution, Rogosa SL agar (Himedia, India) supplemented with 1.32 ml of glacial acetic acid and m Enterococcus agar (Himedia, India) supplemented with 10% aqueous solution of sodium carbonate. After incubation at 37°C (Aerobically for MSB and anaerobically for m Enterococcus agar and Rogosa SL agar) for 48 hours, visible colonies were counted. The counts were made of colonies with morphologic characteristics of the three oral bacteria on the plates using a magnifying lens and were expressed as number of colony forming units (CFU) per/ml of saliva. All of analysis was carried out using SPSS software Ver.19 (SPSS Co. Ltd Tokyo, Japan)

Results

Thirty three children ranging in age from 4-9 years were included in the study. Mean dmft of examined children was 6.3. The dmft score for this random sample is very high comparing to other scores in developed and even developing countries while mean for pH was neutral about 7.6 (table 1).

Table 1: statistical values of dmft, pH, Streptococci mutans, oral lactobacilli and oral enterococci

	dmft	pH	Streptococci mutans	Oral lactobacilli	Oral enterococci
Mean	6.3030	7.6212	7×10^6	6×10^8	2.3×10^9
Std. Deviation	4.40515	.63775	8.5×10^6	1.2×10^9	4×10^9
Minimum	.00	6.00	.00	.00	2×10^8
Maximum	15.00	9.00	3.7×10^7	4×10^9	2.5×10^{10}

Correlations of salivary level of Streptococci mutans, oral lactobacilli and enterococci with and salivary pH and dmft were determined. Positive correlation was observed between dmft and Streptococci mutans ($p=0.553$).

Table 2: Correlation between dmft and Streptococci mutans

	dmft	Streptococci mutans
Pearson Correlation	1	.553**
Sig. (2-tailed)		.001
N	33	33
Pearson Correlation	.553**	1
Sig. (2-tailed)	.001	
N	33	33

** Correlation is significant at the 0.01 level (2-tailed)

No significant relations were founded between dmft with oral lactobacilli, oral enterococci and between three bacteria and pH; Table 3, 4, 5 respectively.

Table 3: Correlation between dmft and Oral lactobacilli

	pH	Streptococci mutans	Oral Enterococcus	Oral Lactobacilli
Pearson Correlation	1	-.144	-.057	.090
Sig. (2-tailed)		.425	.751	.618
N	33	33	33	33

Table 4: Correlation between dmft and Oral enterococci

		dmft	Oral enterococci
dmft	Pearson Correlation Sig. (2-tailed)	1	-.156 .385
Oral Enterococci	Pearson Correlation Sig.(2-tailed)	-.156 .385	1

Table 5: Correlation between Salivary pH and the Three bacteria

	pH	Streptococci mutans	Oral Enterococcus	Oral Lactobacilli
Pearson Correlation	1	-.144	-.057	.090
Sig. (2-tailed)		.425	.751	.618
N	33	33	33	33

Discussion

Dental caries remains one of the most common chronic infectious childhood diseases and individuals remain susceptible to the disease throughout their lifetime⁽¹⁵⁾. The dmft score for this random sample was very high compared to other scores in developed and even developing countries. A study was done by Marthaler⁽¹⁶⁾ in several developing countries in children aged 5-7 years, showed average dmft values below 2.0 for Denmark, England, Finland, Italy, Netherlands and Norway. Even the higher dmft values which were reported in other developing countries like Belarus 4.7⁽¹⁷⁾ Hungary 4.5,⁽¹⁸⁾ and Romania 4.3⁽¹⁹⁾ still higher than this dmft score. Important explanation for this result is due to higher and uncontrolled consumption of sugar among this age group and lack of sufficient dental treatments. Many cross-sectional studies had shown a relationship between sugars consumption and levels of caries in the primary and/or permanent dentitions in countries or areas throughout the world like Saudi Arabia⁽²⁰⁾. The positive relation between dmft and *S. mutans* was accepted by a lot of researches which found out and proved the influence of salivary *S. mutans* on caries experience in children population⁽⁸⁾. The key virulence factors for *S. mutans* are acidogenicity, acid tolerance and synthesis of water insoluble glucan from sucrose⁽²¹⁾.

The mean number of *S. mutans* per ml unstimulated saliva in this study was about 7×10^4 which is not between 10^5 and 10^6 to be accepted by Krasse⁽²²⁾ as risk for caries in adults and older children. Contrasting to this study, several studies have failed to detect *S. mutans* in a significant portion of the children who developed caries suggesting that additional species may played a significant role in dental caries development in children⁽²³⁾. In the same line, a study by Dasanayake⁽²⁴⁾ using "Dentocult MS" set found no positive correlation between caries and salivary MS level in children of 2-17 year olds. Failure to correlate lactobacilli count with dmft as occur in this study may indicate that lactobacilli acting alone may not initiate caries, but that it may initiate caries possibly by increasing the acid production in plaque⁽²⁵⁾. Some lactobacilli are cariogenic in experimental animals and their cariogenicity is dependent upon consumption of carbohydrate rich diets of animals⁽²⁶⁾.

Lactobacillus monitoring using saliva has less uncertainty of interpretation than saliva monitoring for *S. mutans* probably because lactobacilli are mucosal colonizers, not tooth colonizers. Lactobacillus colonies may substantially reflect salivary contamination of that tooth surface⁽⁹⁾. Lactobacilli do not avidly colonize the teeth and may be transiently found in the mouth before the teeth erupt; they preferentially colonize the dorsum of the tongue and are carried into saliva by the sloughing of the tongue's epithelium⁽²⁷⁾. Marsh⁽²⁸⁾ founded that lactobacilli were rarely isolated in a cross-sectional epidemiological study which had been undertaken to relate the bacterial composition of approximal dental plaque with the earliest stages of caries development in school-children but other authors reported the presence of lactobacilli in 100% of sampled children⁽²⁹⁾. Nevertheless, a strong correlation has been established between the lactobacillus count and caries⁽³⁰⁾. The higher the dmf index, the higher the number of children harbouring a high lactobacillus count⁽³¹⁾. *E. faecalis*, a gram positive cocci is associated with various periradicular diseases including primary endodontic infections, persistent

infections and asymptomatic chronic periradicular lesions. It can also survive by genetic polymorphism and its ability to bind to dentin, invade dentinal tubules, and survive starvation⁽³²⁾.

Although Souto⁽¹³⁾ isolated *E. faecalis* from caries and lesions associated with periodontal diseases, no correlation was founded between dmft and this microorganism in this study. Studies have shown that *E. faecalis* contributes to the failure of endodontic therapy while in vitro studies, it had been shown to invade dentinal tubules⁽³³⁾. The ability of *E. faecalis* to tolerate or adapt to harsh environmental conditions may act as an advantage over other species. It may explain its survival in root canal infections⁽³⁴⁾. So, large number of this microorganism can be present with one badly necrotic carious tooth exceeding the number present with several simple carious teeth making the correlation between dmft and oral enterococci fruitless. The pH of saliva is an important component to maintain the integrity of oral mucosa⁽³⁵⁾. The pH below which enamel dissolves is not constant but rather is inversely proportional to the concentrations of calcium and phosphate in the saliva and plaque fluid⁽³⁶⁾ so it differs from person to other. The pH value of saliva differs according to chemical composition and diet i.e. in people with low salivary concentrations of calcium and phosphate, the critical pH may be 6.5, whereas in those with high salivary calcium and phosphate concentrations, it may be 5.5⁽³⁷⁾ so there is wide range in pH of saliva from 6-9 with mean about 7.6. Sullivan⁽³⁸⁾ concluded that saliva's pH has no influence on caries experience in different age groups which coincide with the absence of relation between the three bacteria and salivary pH.

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