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Salivary pH, Flow Rate and *Streptococcus mutans* Count in Relation to Oral Health Statusamong Colored Eyes Adolescentsin Baghdad/Iraq

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Differences in susceptibility to dental caries occurs even under the similar, controlled conditionsbecause of genetic variations, certain environmentalfactors are potentially more cariogenic for some individuals than for others. Salivary constituents differences may cause variation in caries susceptibility. This study was conducted to assess the salivary pH, flow rate and streptococcus mutans count in relation to oral health status among colored eyes adolescents. The study group included all 85 colored eyes adolescents from 6 secondary schools, while control group contained 85 brown eyes adolescents selected randomly from the same schools (12-15 for both groups, only males). Decayed, missing and filled teeth (DMFT), plaque (PlI), Gingival (GI) and calculus (CI) indices were used to measure oral health status for both groups.Salivary samples collection was done in the morning at least one hour after breakfast, then normal saline was added to have tenfold dilutions, to assess thetotal colonycounting of the caries related microorganisms (*streptococcus mutans*), after that inoculation was done inthe special selective media (Mitis-Salivarius-Bacitracin agar). Counting of bacterial colonies were estimated by the aid of dissection microscope. *Salivary pH and flow rate were measured directly. The data of present study was analyzed using SPSS version 21.* The median and mean rank values for DMFT, PlI, GI and CI indices were higher in study group than control group with high significant differences with regardto DMFT and PlI.A strong positive correlation was recorded between DMFT and PlI, GI, CI in both groups. Salivary pH and flow rate were lower in study compared to the control group (statistically significant difference with salivary flow rate). Higher mean value of salivary streptococcus mutans count among study group compared to control group with statistical significant difference. Dental caries experience and *streptococcus mutans* count were higher in colored eyes adolescents than brown eyes adolescents. Oral hygiene and salivary flow rate were lower in study group than control group.

Keywords: DMFT, PlI, *Streptococcus mutans*.

Dental caries refers to the localized destruction of susceptible dental hard tissues by acidic by-products from the bacterial fermentation of dietary carbohydrates[1]. which results from an ecological imbalance in the equilibrium between tooth minerals and oral biofilms (plaque)[2]. The biofilm is characterised by microbial activity, resulting in fluctuations in plaque pH. This is a result of both bacterial acid production and buffering action from saliva and the surrounding tooth structure. The tooth surface is therefore in a dynamic equilibrium with its surrounding

environment. As the pH falls below a critical value, the demineralisation of enamel, dentine or cementum occurs, while a gain of mineral (remineralisation) occurs as the pH increases[3].[1]

Dental caries is a multifactorial disease. Environmental risk factors—such as dental plaque, cariogenic diet, insufficient fluoride exposure, poor oral hygiene, high numbers of cariogenic bacteria, and inadequate saliva flow—may influence the development of dental caries[4,5]. 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[6]. *Steptococcus mutans*, a member of the oral micro flora, is considered to be the primary causative agent of dental caries (or tooth decay) and is one of the best known biofilm forming bacterium[7]. There is a direct relation between streptococcus mutans levels in saliva and the number of colonized tooth sites and to their proportion in dental plaque[6,8]. However, when exposed to the same levels of environmental risk factors, some patients may be more susceptible or resistant to caries than others. Those differences may be due to genetic factors in the etiopathogenesis of dental caries[9,10].

To date, most genetic studies analyzed the problem of detecting a genetic factor contributing to caries by testing genetic variation, such as single-nucleotide polymorphisms (SNPs) in specific genes, for an association between variants at a genetic locus and caries. These genes can be grouped into categories based on the factor influencing dental caries. The major candidate gene categories to date include enamel formation genes, immune response genes, genes related to saliva, and genes related to taste and dietary habits (Vieira *et al*. 2014)[11]. This study was designed to assess the salivary pH, flow rate and *streptococcus mutans* count in relation to oral health status among colored eyes adolescents.

MATERIALS AND METHODS

From six secondary schools, all colored eyes adolescents (eighty five) were included as study group while eighty five brown eyes adolescents selected randomly from the same schools as control group, with age range(12-15 for both groups, only males). All adolescents in both groups,caries experience measured through the application of decayed, missing and filled teeth index (DMFT) for permanent teeth according to criteria of WHO[12]. Oral hygiene status evaluated by application plaque index (PlI) of Silness and Löe[13], and calculus index (CI) of Ramfjord[14]. Gingival inflammation assessed by using Gingival Index (GI) of Löe and Silness[15].

For salivary samples, each child was asked to sit down and relaxes much as possible and asked to chew a piece of Arabic gum for one minute before all the saliva was removed by expectoration; chewing was then continued for ten minutes with the same piece of gum and the collection of saliva by spitting was done during this time[16]. Salivary flow rate assessed immediately by dividing the total volume of saliva collected in milliliter on the time of collection in minute. pH of saliva measured by using electronic pH meter.

After collection of the saliva, dilution was performed with normal saline. After that saliva was applied on the surface of the selective media by using micropipette (Mitis salivaris -Bacitracin agar medium are the selective medium for *mutans streptococci*). Afterincubation of the plates in an anaerobicatmosphere for 48 hours at 37°C, counting ofCFU (colony forming units) with morphologycharacteristic of *streptococcus* mutans (numbers of CFU per milliliter of saliva)[17].

By using SPSS 21 version (Statistical Package for Social Sciences), frequency distribution for selected variables was done first. The statistical significance, direction and strength of linear correlation between two quantitative normally variables, one of which being non-normally distributed was measured by Spearman's rank linear correlation coefficient. P value less than the 0.05 level of significance was considered statistically significant. All analyzed tests were bilateral.

RESULTS

The scoring of DMFT index was carried out in both groups. Table 1 and Table 2 show caries-experience (median and mean rank of DMFT) among study and control groups. The median ad mean rank values for DMFT were higher in study group than control group. Mann-

Whitney test (p-value < 0.05) was used to compare between study and control groups. The result recorded a highly significant difference between the mean rank DMFT at study group when compared to the control group. For DMFT (Mann-Whitney value = 2359.500, Z = -3.941), as p-value <0.001 for the difference.

Table 3 illustrates median and mean rank values of plaque, gingival and calculus indices among study and control groups. The median and mean rank values of plaque, gingival and calculus indices in study group were found to be higher than control group with highly significant differences for plaque index (Mann-Whitney value = 2636.000, Z = -3.046, p-value = 0.002), while no significant differences in relation to gingival and calculus indices.

The correlation coefficient between caries-experience of permanent teeth with PlI, GI and CI among study and control group is seen in

Table 1. Caries-experience (median) of DMFT of permanent teeth among study and control groups.

Groups	No.	DMFT								Median
		0	1	2	3	4	5	6	≥7	
Study	85	5	4	9	9	18	16	15	9	4
Control	85	9	12	18	15	11	9	6	5	3

Table 2. Mean rank of DMFT of permanent teeth among study and control groups

	Groups	Mean Rank	Mann-Whitney	z-value	p-value
DMFT	Study	100.24	2359.500	-3.941	<0.001**
	Control	70.76			

**Highly Significant

Table 3. Median and mean rank of plaque, gingival and calculus indices among study and control groups

Variables	Groups	Median	Mean Rank	Mann-Whitney	z-value	p-value
PI	Study	0.92	96.99	2636.000	-3.046	0.002**
	Control	0.58	74.01			
GI	Study	0.33	88.81	3331.500	-0.877	0.380
	Control	0.29	82.19			
CI	Study	0.08	85.20	3587.000	-0.085	0.932
	Control	0.00	85.80			

** Highly Significant

Table 4. Correlation coefficient between caries-experience of permanent teeth and plaque, gingival and calculus indices among study and control groups

	Groups	PII		GI		CI	
		r	p	R	p	r	P
DMFT	Study	0.762	< 0.001**	0.761	< 0.001**	0.723	< 0.001**
	Control	0.914	<0.001**	0.725	<0.001**	0.850	<0.001**

** Highly Significant

Table 4. A strong positive correlation was recorded between DMFT and PlI, GI, CI in both groups with high significant differences.

Table 5 shows mean values of pH and flow rate (ml/min) in stimulated saliva among study and control groups. A lower value of salivary pH and flow rate were found among study compared to the control group, which was statistically significant with salivary flow rate (p-value = 0.024, t-value = -5.547, df = 168), while no significant difference with salivary pH.

The mean values of salivary streptococcus mutans count among study and control groups is illustrated in Table 6. The result shows a higher mean value of salivary *streptococcus mutans* count among study group in contrast to control group with statistical significant difference (p-value = 0.029, t-value = 5.500, d.f = 168).

The correlation coefficient between salivary *streptococcus mutans* count with salivary pH and flow rate among study and control group is shown in Table 7. A weak negative correlation was recorded between salivary *streptococcus mutans* count with salivary pH and flow rate in both groups with statistical significant difference regarding control group only (p-value = 0.019).

DISCUSSION

Genes are the building blocks of human growth and development. They determine many characteristics, like hair and eye color. Genes also affect the way the immune system functions or how it responds to threats.Evidence of a genetic contribution to caries is based on: immune response , sugar metabolism and consumption, dental hard

Table 5. Mean values of salivary pH and flow rate among study and control groups with statistical difference

Salivary parameters	Groups	(Mean ± SD)	Statistical difference t-value	df	p-value
pH	Study	6.89 ± 0.56	-4.209	168	0.886
	Control	7.25 ± 0.54			
Flow rate(ml/min)	Study	1.52 ± 0.30	-5.547	168	0.024*
	Control	1.75 ± 0.24			

* Significant

Table 6. Mean values of salivary *streptococcus mutans* count among study and control groups with statistical difference

Groups	No.	Streptococcus mutans CFU ($\times 10^4$/ml)(Mean ± SD)	Statistical difference t-value	df	p-value
Study	85	8.05 ± 2.63	5.500	168	0.029*
Control	85	6.07 ± 2.03			

* Significant

Table 7. Correlation coefficient between salivary streptococcus mutans count with salivary pH and flow rate among study and control groups

	Groups	pH r	pH p	Flow Rate r	Flow Rate p
Streptococcus mutans Counts	Study	-0.019	0.863	-0.026	0.812
	Control	-0.28	0.801	-0.25	0.019

tissue and salivary flow, salivary constituents and salivary defense systems[18].

One aspect of genetic effects is modification in immune response. Human leukocyte antigen (HLA) or major histocompatibility complex (MHC) molecules have important roles in the immune responsiveness which is controlled by genes on the short arm of chromosome 6. Polymorphism in MHC molecules may cause some variations in immune responses against oral colonization levels between individuals and may influence an individual's susceptibility to caries[19,20].

In this study, the variation on oral health between colored eyes adolescents and brown eyes adolescents may attribute to differences in: inherited immune response, salivary *streptococcus mutans* counts, salivary flow rate and salivary constituents between two groups.There are no previous studies similar to this study to compare with. Further studies are needed to establish the exact factors responsible for these results.

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