

## Dental Caries in Relation to Salivary Factors in Saudi Population Groups

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### Abstract

**Aims:** The aim of this cross-sectional study was to register caries trends in relation to some risk factors such as oral hygiene, salivary level of streptococcus mutans (SM), lactobacilli (LB), yeast, salivary flow rate, buffering capacity, pH, and fluoride level among different age groups of a Saudi population. The study also aimed at determining which of the salivary factors correlate significantly to caries.

**Methods and Materials:** A random sample of 312 subjects belonging to age groups six-11, 12-17, and 18-40 years were selected from patients attending the screening dental clinics of the Faculty of Dentistry, King Abdulaziz University in Jeddah, Saudi Arabia. Patients were examined clinically to measure the caries experience using decayed, missing, and filled teeth (DMFT) and oral hygiene levels using the Green and Vermillion method. Resting and stimulated saliva were collected to determine flow rate, fluoride, pH, buffering capacity as well as salivary level of SM, LB and yeast.

**Results:** The mean DMFT was 7.59. Females as well as the older age group of patients were more affected than males and younger patients. The only salivary factor showing a significant relationship to caries was pH. Counts of SM and LB correlated positively with DMFT scores. Significant higher plaque scores were present among the highest caries level group.

**Conclusion:** The results stress the importance of screening for caries risk factors. The screening starts with simple clinical observations, expanding to a diversified pattern of tests to assess the pH level and SM and LB counts designed to target high-risk subjects who should be given the most intensive caries preventive measures.

**Keywords:** Caries, salivary flow rate, pH, buffering capacity, microorganisms, oral hygiene

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## Introduction

Although the prevalence of dental caries has declined markedly over the last 20 years in most countries in the Western world, the disease is still a major problem for both adult and children especially in developed countries.<sup>1,2</sup> However, recent epidemiologic studies indicate the decline in caries in the Western region has not continued during the 1990s and may have leveled out.<sup>3,4</sup>

Data from the Saudi population clearly show caries continues to be a major problem. Dental caries increases markedly with age,<sup>5,6</sup> and there is a need for an improved approach for prevention and therapy.

The factors related to the development of dental caries are extremely relevant in the disease process. The microorganisms with cariogenic capacity do not determine the presence of dental caries. It is necessary to have suitable substrates and physiological conditions in the host to allow implantation and survival of these microorganisms in order to facilitate the development of caries. That is why caries is considered to be a multifactorial disease.

The salivary tests most often used for clinical purposes are flow rate of unstimulated and stimulated saliva, buffering capacity, and growth of colony-forming microorganisms and in most cases counts of lactobacilli (LB), mutans streptococci, and yeast.<sup>7,8</sup>

Because dental caries is not a specific infection microbial tests are problematic. There are many people with one or more of the most potent cariogenic micro-organisms in their oral cavity without any sign of a caries attack. At the same time, an abundance of carious lesions occur in many patients in the absence of high levels of cariogenic bacteria.<sup>8,9</sup> The role of oral hygiene in dental caries is unclear. Although tooth brushing is strongly recommended in official dental health education materials and by oral health professionals as one of the principle methods of eliminating dental plaque and preventing gingivitis and dental caries, studies have provided conflicting evidence.<sup>10</sup> This highlights the need for a strategy including a combination of diagnostic tests and indices to target high caries risk groups.

The aims of this study were to register caries trends in relation to some known risk factors including oral hygiene, salivary level of mutans streptococci, LB, yeast, salivary flow rate, buffering capacity, pH, and fluoride level among Saudi groups of different ages and to determine which of the factors correlate significantly to caries.

## Methods and Materials

### Subjects

Three hundred twelve Saudis were randomly selected from patients attending screening dental clinics at the School of Dentistry at King Abdulaziz University in Jeddeh, Saudi Arabia. The subjects were divided into three age groups, six-11, 12-17, and 18-40 years.

The study was approved by the Ethics Committee of the University, and all the patients conveyed written informed consent.

### Data Collection and Calibration

The subjects underwent a clinical examination, sialometry, and analysis of the saliva. Data were collected by two dentists who were calibrated using a series of training sessions designed to standardize data collection technique to achieve good levels of intra-and inter-examiners reliability prior to the start of the study. The reliability levels varied from 0.81 to 0.92 using Cohen's Kappa statistics.

### Clinical Examination

Caries level and oral hygiene status were recorded. Determination of decayed, missing, and filled teeth (DMFT) for permanent teeth was done by visual examination and probing according to the criteria set by the World Health Organization (WHO).<sup>11</sup> Oral hygiene was recorded according to the amount of plaque accumulation using the criteria established by Green and Vermillion.<sup>12</sup>

### Saliva Analysis

#### Flow Rate

Saliva samples were collected from subjects under close supervision no earlier than two hours after a meal between 9:00 am and 12:00



noon. Prior to collection of each sample subjects were asked to sit and relax. Resting saliva was collected first for five minutes followed by a collection of paraffin-stimulated saliva for an additional five minutes. The saliva was collected in a graduated sampling tube fitted with a funnel to facilitate the collection process.

### **The pH**

The pH of the collected stimulated and unstimulated saliva was measured, immediately after collection, using an EC 40 Benchtop pH / ISE meter (Hach Company, Loveland, CO, USA). A volume of 0.1 ml of saliva was dropped onto the pH-sensitive electrode to measure the pH of the saliva. The digital reading was allowed to stabilize for a few seconds before the pH reading was taken. Autocalibration is a feature of the EC40 meter that automatically recognizes five pH buffers: 1.68, 4.01, 7.00, 10.01, and 12.46, within a range of  $\pm 0.5$  pH units. The accuracy of the pH meter was checked at regular intervals to ensure the readings were correct.

### **Fluoride Level**

Fluoride level in the collected samples was determined with the use of a Hach Model 50265 fluoride electrode (Hach Company, Loveland, CO, USA). The fluoride content (ppm) was calculated using a standard curve created from the analysis of standardized solutions collected at the same time as the saliva samples. The standardized solutions were made from 1mg/L and 10mg/LF- to which fluoride Total Ionic Strength Adjustment Buffer Powder was added.

Fluoride level calibration was done using the manufacturer's recommendation. A two point standard calibration was performed at the beginning of each day saliva samples were tested. This procedure determined the electrode slope, confirmed the electrode was working

properly, and stored the slope value in the memory of the Hach Model 50265 testing device.

### **Buffering Capacity**

Saliva buffering capacity was assessed immediately after the collection using a commercial Dentobuff strip test (Orion Diagnostica, Espoo, Finland). The buffer effect was determined by comparing visually the color changes in the Dentobuff strip employing the manufacturer's color chart. The buffering capacity was rated as low, intermediate, or high.

### **Microbial Composition**

Salivary counts of streptococcus mutans (SM) and LB were determined from resting and stimulated saliva using commercial techniques with Dentocuff-SM and the Dentocult-LB (Orion Diagnostica, Espoo, Finland).<sup>13</sup> The classification of test scores 1 and 2 correspond to  $<10^5$  and  $\geq 10^5$  colony forming units (CFU)/ml, respectively.

To record the presence of yeast, the saliva samples were vortexed (Vortex, Scientific Industries Inc., Springfield, NY, USA) for 1 min and then serially diluted ten-fold to  $10^{-2}$  in Reduced Transport Fluid (RTF). From the undiluted sample and the dilutions, 50 $\mu$ L aliquots were spread on Sabouraud dextrose agar (SAB) plates (Oxoid Ltd, Basingstoke, UK) using a sterile glass spreader. The plates were incubated at 30°C for three days. Yeast colonies were recorded as present or absent. Fifteen saliva samples were excluded from yeast analysis due to either a small amount of saliva collected or contamination of the samples.

### **Statistical Analysis**

Statistical analysis was performed using SPSS software (SPSS, Inc., Chicago, IL, USA). To analyze the DMFT in relation to salivary factors and oral hygiene scores the analysis of variance (ANOVA) test was used. The Chi-square test was used to investigate the association of the DMFT with microbial flora and the buffering capacity of saliva. A P value of  $<0.05$  was used to identify statistically significant differences.

### **Results**

Three hundred twelve subjects of both sexes were examined (42.6% female and 57.4% males). The mean of DMFT scores for the sample was

7.59 per patient. A higher mean score was registered in females (8.36) than males (6.56). The gender difference was statistically significant. The mean DMFT in children six-11 years of age was 2.93. This score increased with age to a value of 6.83 in the 12-17 year-old age group and reached the highest level (12.51) in the 18-40 year-old age group. Age difference was statistically significant. Table 1 presents the distribution of caries (DMFT) according to sex and age.

The DMFT scores for the sample varied from zero to 14. Only 19 subjects (0.06%) were caries-free, this percentage was too low to make a meaningful comparison between a caries-free and a caries active population. As a result, the sample was dichotomized (DMFT<4, 4-9 and >9) for further analysis to represent subjects with low, medium, and high caries activity, respectively.

Table 2 presents the analysis of flow rate, pH, fluoride level, and buffering capacity in resting saliva in relation to caries levels.

The pH level was the only factor significantly related to the caries level and was the only factor showing a significant relationship with the DMFT when stimulated saliva was used (Table 3).

Table 4 shows the relationship between dental caries and the concentration of LB and SM in resting saliva expressed as the CFU/ml of a microbial species in saliva. The caries level was also analyzed in relation to the presence or absence of yeast. In resting saliva none of the microbial species showed a significant relationship to dental caries.

Table 5 presents the relationship between the levels of microorganisms in stimulated saliva and

**Table 1. Distribution of caries according to sex and age.**

	Sex				Age (years)			
	Male n = 133 Mean (SD)	Female n = 179 Mean (SD)	Total n = 312 Mean (SD)	P Value (t-Test)	6 to 11 n = 114 Mean (SD)	12 to 17 n = 99 Mean (SD)	18 to 40 n = 99 Mean (SD)	P value (Scheffe test)
DMFT	6.56 (5.15)	8.36 (6.28)	7.59 (5.88)	<.01	2.93 (2.29)	6.83 (4.63)	12.51 (5.45)	<.001

**Table 2. Relationship between caries and resting saliva variables.**

DMFT	Flow rate ml/min Mean (SD)	pH Mean (SD)	Fluoride ppm Mean (SD)	Buffering Capacity		
				Low n (%)	Med n (%)	High n (%)
Low < 4 n = 156	2.8.4 (1.894)	7.226 (.394)	.165 (.107)	45 (28.8)	94 (60.3)	17 (10.9)
Medium 4 – 9 n = 48	3.121 (2.016)	7.155 (.434)	.148 (.187)	15 (31.3)	27 (56.3)	6 (12.5)
High > 9 n = 108	3.422 (2.057)	6.926 (.392)	.143 (.183)	35 (32.4)	59 (54.6)	14 (13.0)
P value	NS	.000	NS	NS		

P value using ANOVA except for buffer - Chi square  
NS non significant



**Table 3. Relationship between caries and stimulated saliva variables.**

DMFT	Flow rate ml/min Mean (SD)	PH Mean (SD)	Fluoride ppm Mean (SD)	Buffering Capacity		
				Low n (%)	Medium n (%)	High n (%)
Low < 4 n = 156	7.290 (3.101)	7.509 (.367)	.156 (.158)	2 (1.3)	33 (21.2)	121 (77.6)
Medium 4 – 9 n = 48	7.615 (3.482)	7.385 (.314)	.143 (.187)	1 (2.1)	11 (22.9)	36 (75.0)
High > 9 n = 108	8.364 (3.652)	7.250 (.297)	.140 (.167)	4 (3.7)	27 (25.0)	77 (71.3)
<b>P value</b>	<b>NS</b>	<b>.000</b>	<b>NS</b>	<b>NS</b>		

P value using ANOVA except for buffer - Chi square  
NS non significant

**Table 4. Relationship between caries and microbial flora in resting saliva.**

DMFT	LB		SM		Yeast	
	< 10 <sup>5</sup> n (%)	≥ 10 <sup>5</sup> n (%)	< 10 <sup>5</sup> n (%)	≥ 10 <sup>5</sup> n (%)	Present n (%)	Absent n (%)
Low < 4 n = 156	71 (45.5)	85 (54.5)	72 (46.2)	84 (53.8)	54 (38.3)	87 (61.7)
Medium 4-9 n = 48	17 (35.4)	31 (64.6)	25 (52.1)	23 (47.9)	20 (41.7)	28 (58.3)
High > 9 n = 108	35 (32.4)	73 (67.6)	53 (49.1)	55 (50.9)	44 (40.7)	64 (59.3)
<b>P value *</b>	<b>NS</b>		<b>NS</b>		<b>NS</b>	

\* Using Chi square test  
NS = non significant

**Table 5. Relationship between caries and microbial flora in stimulated saliva.**

DMFT	LB		SM		Yeast	
	< 10 <sup>5</sup> n (%)	≥ 10 <sup>5</sup> n (%)	< 10 <sup>5</sup> n (%)	≥ 10 <sup>5</sup> n (%)	Present n (%)	Absent n (%)
Low < 4 n = 156	68 (43.6)	88 (56.4)	82 (52.5)	74 (47.4)	73 (51.8)	68 (48.2)
Medium 4 – 9 n = 48	13 (27.1)	35 (72.9)	22 (45.8)	26 (54.2)	22 (45.8)	26 (54.2)
High > 9 n = 108	42 (39.3)	66 (60.7)	51 (47.2)	57 (52.8)	52 (48.1)	56 (51.9)
<i>P</i> value *	.025		.041		NS	

\* Using Chi square test  
NS = non significant

**Table 6. Relationship between caries and oral hygiene.**

Caries Activity	Mean Oral Hygiene Score	SD
Low	1.168	.576
Med	1.220	.706
High	1.463	.682
<i>P</i> value	.013	

*P* value using ANOVA

caries. The association between DMFT and LB and SM concentration in stimulated saliva was statistically significant. Analysis revealed a higher percentage of individuals showing medium to high DMFT scores had saliva with levels equivalent to 10<sup>5</sup> LB and SM CFU/ml or more. The presence or absence of yeast was of insignificant value in relation to the caries level.

In the studied population the mean plaque scores were 1.168 in the group having low caries activity. This value increased to 1.463 in the group with the highest caries activity. Severity of caries correlated significantly with the mean plaque index.

### Discussion

Oral disease is a major public health problem due to the high prevalence in all regions of the world and the greatest impact on the socially marginalized populations.<sup>1,14,15</sup> The evaluation of caries risk is important. It gives an opportunity to improve hygiene, diet, and implement preventive measures in an exposed population.

The caries experience in the present sample is considered high (DMFT of 7.59). The mean DMFT scores increased with age. The DMFT values were higher than those reported previously in similar age groups from other populations.<sup>16-18</sup>



Considering the relation between DMFT and gender, the mean of DMFT was higher among females than males. When dental caries rates were reported by gender in the dental literature, females were found to exhibit typically higher prevalence rates than males. This finding is generally true for diverse cultures and for a wide range of chronological periods.<sup>16,19,20</sup> Exceptions exist but they are uncommon.<sup>21,22</sup> A high caries prevalence among females is often explained by frequent snacking during food preparation and hormonal fluctuations during events such as puberty, menstruation, and pregnancy. These environmental variables significantly favor cariogenic flora in women.<sup>19,23</sup>

Comparing salivary factors in subjects with different levels of caries activity revealed pH is the only factor which associates significantly with caries activity. This result contradicts Llenc-Puy et al.<sup>24</sup> who found no significant relation between pH value and dental caries. In their study pH values of <6.5 were recorded as low. However, only a small number of their samples exhibited pH values under 6.5 which might account for the lack of statistical significance of the results obtained. In the present study the mean pH values were used for analysis and not a cut point in the data since the critical pH below which enamel dissolves is not constant but rather inversely proportional to the concentrations of calcium and phosphate in the saliva and plaque fluid.<sup>25</sup>

The natural repair process, or remineralization, occurs when the pH rises and calcium and phosphate from saliva together with fluoride enter the surface region of the caries lesion. The present findings signal the importance of pH and

should motivate the oral health professionals to consider this factor in order to facilitate the natural tooth repair process.

In the present study salivary flow rates of resting and stimulated saliva were not significantly different among the three caries groups. This finding points out a high salivary flow rate is an irrelevant factor in an individual's resistance to caries. Similar results have been reported by other studies.<sup>26,27</sup> In contrast, some studies confirmed the importance of salivary output in maintaining a healthy oral environment,<sup>28</sup> specifically caries.<sup>29</sup>

The fluoride level in stimulated saliva of the study population varied between 0.140 and 0.156 in high and low caries activity groups, respectively. Laboratory studies focusing on demineralization and remineralization aspects of the caries process showed as the fluoride concentration increased remineralization increased with an optimum being achieved at approximately 0.08 ppm.<sup>30,31</sup>

Clinical studies reported mean baseline fluoride concentrations in saliva of 0.04 to 0.07.<sup>32,33</sup> The high level of fluoride in saliva of the present sample could be related to environmental factors such as increased water consumption due to hot weather or an additional source of fluoride in their food. However, further investigations are required to determine the underlying sources.

The fluoride concentration in whole saliva from the present study was of insignificant value in relation to the caries level. Although fluoride levels in saliva were considered too low to be of any relevance to the development of dental caries,<sup>34</sup> there are some studies which showed one of the major cariogenic effects of fluoride is its presence in low concentrations in liquid phase surrounding the teeth.<sup>35,36</sup>

When the buffering capacity was evaluated, no significant relevance was found between this variable and caries levels as a protector factor against caries. Most of the study population had high buffer capacity of stimulated saliva regardless of their caries status.

Microbial salivary tests have been recommended for mass screenings of entire youth populations. However, salivary tests have seldom been

applied in everyday dental care.<sup>37,38</sup> Evaluation of microbial salivary tests has shown large intra-individual variation,<sup>39,40</sup> making these variables unreliable in describing caries-related oral ecology. Further evaluations of these tests have shown a weak to only a moderate ability to predict future caries experience.<sup>41-43</sup> This factor has not been encouraging to oral health professionals to use this measure in their practices.

Thenisch et al.,<sup>9</sup> in their systemic review to find the usefulness of bacterial testing in caries risk assessment, reported the presence of mutans streptococci, both in plaque or saliva of young caries-free children, appears to be associated with a considerable increase in caries risk. However, lack of adjustment for potential confounders in the original studies limits the extent to which interpretations for practice can be made.

The presence of SM and LB in saliva with values  $10^5$  CFU/ml was found in most of the study samples. A significantly higher percentage of individuals in the high caries group demonstrated these high levels. These findings are in agreement with some previous studies<sup>40,44</sup> and still provide contradiction to others.<sup>42,45</sup>

The presence or absence of yeast was irrelevant to the caries levels in the present study. This is in contrast to that reported by Akdeniz et al.<sup>46</sup>

who found candidal prevalence to be higher in children with caries than those who are caries free. Although tests for identification of bacterial strains is considered an expensive measure and too high to perform for all patients,<sup>21</sup> the tests are still considered a valuable measure to be used for high caries risk patients based on the present findings.

The oral hygiene index was recorded to determine if the degree of oral cleanliness is a good measure of future caries activity. However, epidemiological evidence of the relationship between plaque and caries lacks consistency leaving this relationship to caries initiation in doubt.<sup>47</sup> Similar to Peterson et al.<sup>48</sup> the present study findings revealed higher plaque score as a factor contributing significantly to the higher risk profiles for the study population. The exact role oral hygiene plays as an initiating factor is not yet well documented.

### Conclusion

Based on the findings of the present study, a caries prevention strategy based on multiple screening phases that includes simple clinical assessment and a diversified pattern of tests, which assess pH level and SM and LB counts, is suggested. This strategy would serve to identify high-risk subjects who should be given the most intensive caries preventive measures to prevent future caries especially in children with special needs.



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