

Correlating dental caries with oral bacteria and the buffering capacity of saliva in children in Madinah, Saudi Arabia

Ahmed Bhayat, Mohammad Sami Ahmad, Tamer Hifnawy^{1,2},
Mohammed Saad Mahrous², Hisham Al-Shorman, Layla Abu-Naba'a³, Hala Bakeer

Departments of Preventive Dental Sciences, ¹Public Health, Faculty of Medicine Beni Suef University, Egypt, and ²Medical Education, ³Substitutive Dental Sciences, College of Dentistry, Taibah University, Madinah, Saudi Arabia

Corresponding author (email: <ahmedbhayat6@gmail.com>)

Dr. Ahmed Bhayat, Department of Preventive Dental Sciences, College of Dentistry, Taibah University, Madinah, Saudi Arabia.

Abstract

Introduction: Caries is associated with high counts of mutans streptococci (MS), lactobacillus (LB), and a low saliva buffering capacity (BC). No study using odds ratios (OR) has correlated caries and these factors and no similar study has been done in Saudi Arabia before. **Objectives:** To determine: The prevalence of caries, the number of colony forming units (CFUs) of MS and LB, the saliva buffering capacity (BC) and the relationship between these factors. **Materials and Methods:** This was an analytical cross sectional study on children from Madinah. Caries was recorded using dmft/DMFT (decayed, missing, filled teeth). The scores for MS and LB and the BC of saliva was calculated using the Caries Risk Test (CRT)[®]. **Results:** A total of 316 students were examined; two-thirds (62%) were female and 25% were caries free. Saliva was collected from 235 participants and the majority had high MS and LB scores (66 and 71%, respectively) while 25% had a low saliva BC. The odds for those who had high LB and MS CFUs, were 9 and 4 times more at risk to developing dental caries and those with a low BC had significantly more caries ($P = 0.03$). The likelihood for those having severe caries and high counts of LB and MS was 25 ($P < 0.01$) and 6 ($P = 0.042$) times greater, respectively, compared to those with no or mild caries. Those with multiple risk factors were more likely to have caries compared to those with single or no risk factors present. **Conclusions:** The prevalence of caries was relatively high and many respondents had greater than 10^3 CFUs/ml of MS and LB. Almost all with a low BC had severe caries. There was a strong correlation between high MS, high LB, and low BC and the high prevalence of caries; hence the combination of these factors could be confidently used to predict caries in this population.

Key words: Bacteria, buffering capacity, caries

INTRODUCTION

Dental caries is one of the most common chronic diseases affecting millions of people globally.^[1,2] It is a major cause of tooth loss, pain and discomfort worldwide; especially amongst children.^[3] One of

the causative factors for this disease is dental plaque which is the bacterial deposit that forms continuously on the tooth surfaces. The frequency of consumption of soft drinks and the frequency of intake of sweets as risk indicators have also been identified previously in various caries risk assessment models.^[4] It is well-recognized that these are important and essential variables in caries initiation. Thus one may predict a child who is likely susceptible to develop caries using these and other innovative risk factors. Factors such as bacterial counts of mutans streptococci (MS) and lactobacillus (LB) and the buffering capacity (BC) of saliva have been shown to be strong risk factors associated with the initiation and progression of dental caries.^[5,6]

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To prevent dental caries, the reduction of high counts of bacteria, an improvement in oral hygiene and a high BC of saliva would be necessary. One way of detecting bacterial counts and the BC of saliva is by using a caries risk test (CRT) such as the one developed by Ivoclar Vivadent.

It is currently accepted that MS and LB^[5] are instrumental in the cause and progression of dental caries. MS plays a decisive role in the onset of caries, while LB contributes to the progression of caries. Therefore, the presence of these bacteria could predict high risk patients and possible preventive procedures could be put in place to prevent or reduce dental caries.

Studies have also shown that the BC of saliva influences the prevalence of dental caries.^[6] Those that have a high BC are more likely to neutralize the pH and hence have a lower prevalence of dental caries.

The identification of high risk children is essential for planning of oral health treatment and prevention of future diseases. It will also help tertiary institutions when planning outreach activities to focus on communities that are most vulnerable to dental caries, one of the pillars for the success of a community service program.^[7]

However, this study is unique in that it is the first to use the Ivoclar CRT buffer and bacteria kits in Saudi Arabia. It is also the first study carried out in a Saudi Arabian population to correlate the bacterial counts of MS and LB and the BC of saliva with the presence of dental caries.

MATERIALS AND METHODS

This was a cross-sectional observational study which was carried out on 6 and 12 year-old school children in Madinah. It was conducted between January and March 2012 in four primary schools which were randomly selected from a list of registered schools in Madinah. Principals of each school were contacted and introduced to the study. Consent forms were sent to the parents of all the children who were registered in grades one and six and those who consented, underwent a dental examination and saliva samples were collected from each of them.

Prevalence of caries was recorded using the dmft/DMFT (decayed, missing, filled teeth) index and examinations were carried out in the classroom under florescent lighting with subjects sitting on a regular

chair and using a wooden spatula as described by the World Health Organization (WHO).^[8] There were two calibrated examiners who completed all the dental examinations. A calibration exercise was carried out before going to the schools and the inter- and intraexaminer variability was 95 and 98%, respectively.

The bacterial count and pH of saliva was calculated using the Caries Risk Test (CRT)[®] kit from Ivoclar Vivadent. A previous study^[9] showed that the sensitivity and specificity of tests using an agar growth was equally effective as those using the broth growth and concluded that the results are acceptable for mass screening purposes.

The subjects were asked to chew on a paraffin pellet, which was included in the kit, for 30 s and then expectorate the stimulated whole saliva into a disposable cup. A portion of the saliva was then pipetted onto two agar plates, one for MS and one for LB. The agar plates were incubated for 48 h at 37°C to allow for the growth of the organisms. The number of colonies as measured by colony forming units (CFU) per milliliter of saliva was not actually counted, but a score was given depending on the visual number of CFUs present against a reference model chart provided in the instruction manual. The readings were done by consensus amongst three investigators using the model chart. The colonies were scored as either “low” (less than 10⁵ CFUs/ml) or “high” (greater than 10⁵ CFUs/ml) as per the manufacturer’s instruction.

The remainder of the saliva was utilized to determine the BC of saliva. The saliva was pipetted onto a test strip which was left for 5 min. During this time the strip changed color and the BC category was recorded according to the manufacturer’s instructions. A green color was recorded as a “high BC”, a yellow color indicated “medium BC”, and a blue color meant a “low BC”. Consensus of three investigators was used to determine the scores and categories for the variables.

For further statistical analyses, the study population was divided into groups depending on the presence or absence of dental caries. Those without any dental caries in both dentitions were classified as “healthy” (dmft = 0 and DMFT = 0) and those with one or more decayed tooth in either of the dentitions were classified as “diseased” (dmft ≥ 1 or DMFT ≥ 1).

To correlate the severity of dental caries, those without any decay were classified as “healthy” (dmft/DMFT = 0), those with a total of between 1 and 7 decayed teeth

in both dentitions were classified as “mild caries” (dmft + DMFT between 1 and 7) and those with more than 8 decayed teeth were classified as “severe caries” (dmft + DMFT ≥ 8).

Data analysis

The data was coded and keyed into the Statistical Package for the Social Sciences software version 19 (SPSS Inc, Chicago, IL) used in Windows 7. Descriptive analysis by inferential statistics was done. Percentages, means, and standard deviations (SD) were calculated for qualitative and quantitative data. Chi-square test (χ^2) and Fisher’s exact test were performed to statistically analyze qualitative data. A *P* value of 0.05 was considered to control for alpha (type 1) error.

Ethical consideration

The Research Ethics Committee of the College of Dentistry, Taibah University, approved this study. The consent process was done in two steps; parent consent followed by child assent, any child who refused to participate in this was excluded. Some parents accepted for including their children in the dental examination but refused the section of saliva collection.

All children enrolled in this study were given a full written report about their oral condition and dental hygiene.

RESULTS

A total of 316 students were screened; almost two-thirds were female (*N* = 197; 62%) and over half (54%) of them were 6-year-old. A quarter (25%) of the sample was caries free. The mean dmft/DMFT scores are shown in Table 1.

Saliva samples were collected from a total of 235 students and statistical correlations were done on respondents who had both a dmft/DMFT score and a saliva sample. Of these, the majority had high CFUs of MS and LB (66 and 71%, respectively), 51% had a medium BC, and 25% had a low BC.

The vast majority had high CFU scores of MS and LB and there was a direct proportional increase in bacterial counts and mean dmft/DMFT scores. Although those who had high scores of MS and LB had a higher mean dmft/DMFT compared to those with low counts, there were no significant statistical associations. The only significant association was between 6-year-old subjects with high scores of LB and their mean dmft score. In this cohort, those with high LB scores had a mean dmft

of 4.85 compared to those with low LB counts who had a mean dmft of 1.44 (*P* < 0.01).

Table 2 shows the correlation between the bacterial counts and the presence or absence of dental caries. There was a highly significant relationship between high counts of LB and MS and the presence of dental caries (*P* < 0.01 and *P* < 0.01, respectively). The odds for those who had a high score of LB were four times (*P* < 0.01) greater to develop dental caries compared to those who had a low LB score. Similarly, those who had high MS scores were two times (*P* < 0.01) more likely to have developed dental caries compared to those who had low MS scores.

There was also a statistical correlation between the buffering capacity and the presence of caries. Those with low BC were significantly ($\chi^2 = 6.9, P = 0.03$) more likely to have dental caries compared to those with a medium or high BC.

To describe the severity of caries, the dmft/DMFT was divided into three categories; “healthy” (dmft and DMFT = 0); those with mild caries (dmft + DMFT

Table 1: Mean dmft/DMFT scores (N=316)

	d	m	f	Total 1	D	M	F	Total 2
6 years (N=171)	4.48	0.17	0.21	4.86±4.17	0.07	0.0	0.0	0.07±0.34
12 years (N=145)	1.06	0.10	0.24	1.41±2.56	0.90	0.05	0.36	1.31±1.20

dmft/DMFT = Decayed, missing, filled teeth

Table 2: The correlation between dental caries, bacterial counts, and the buffering capacity of saliva (N=235)

Variable	Category	Disease (%)	Healthy (%)	Total
LB score	High CFUs	139 (84)	27 (16)	166
	Low CFUs	37 (54)	32 (46)	69
	Total	176 (75)	59 (25)	235
OR=4.45, P<0.01 (CI=2.4-8.3)				
MS score	High CFUs	126 (81)	30 (19)	156
	Low CFUs	50 (63)	29 (37)	79
	Total	176 (75)	59 (25)	235
OR=2.4, P<0.01 (CI=1.3-4.3)				
Saliva BC	Low BC	25 (93)	2 (7)	27
	Medium BC	92 (76)	29 (24)	121
	High BC	59 (68)	28 (32)	87
	Total	176 (75)	59 (25)	235

$\chi^2=6.9$ at 2 df; *P*=0.03

LB = Lactobacillus, MS = Mutans streptococci, BC = Buffering capacity, CFU = Colony forming units, OR = Odds ratio, CI = Confidence interval, df = Degrees of freedom

between and including 1 and 7), and those with severe caries (dmft + DMFT ≥ 8). These categories were then correlated with bacterial scores which showed a strong correlation between high scores and severe caries ($P = 0.04$ and $P < 0.01$) for both MS and LB, respectively as shown in Table 3. The likelihood ratio for both MS and LB was extremely high; those who had severe dental caries were 25 times ($P < 0.01$) more likely to have high counts of LB compared to those with mild or no caries. Those with severe caries were also 6 times ($P = 0.04$) more likely to have high counts of MS compared to those with mild or no caries.

To predict the risk of dental caries the variables were correlated individually and in different combinations to determine those risk factors which would yield the highest risk for developing dental caries. The results are displayed in Table 4. The odds ratios (OR) varied from 2 to 9 and those with multiple factors were greater at risk of having dental caries compared to those with no factors and those with fewer factors. Those with high

MS counts and low BC were nine times ($P < 0.01$) more likely to have dental caries compared to the rest and those with high MS, high LB, and low BC were eight times ($P = 0.02$) more likely to have dental caries compared to the rest of the subjects.

DISCUSSION

This study was unique as it used three risk factors singly and in combination to predict dental caries. No other study of this sort has been done in Saudi Arabia and the results could confidently be used to classify children from this community into high or low caries risk subjects.

There were more females in the study even though an equal number of boys and girls were asked to participate. A reason was that many males did not provide written consent and hence they had to be excluded. Another reason for the low number of males was due to them being absent on the days that were allocated for the dental screenings. As a result, there were higher numbers of females compared to males. Although 316 students were screened, only 235 provided saliva samples. One of the reasons was that some of the participants could not expectorate sufficient amounts of saliva for the saliva tests to be conducted. In addition, some parents refused consent for saliva testing and did not want their children to provide saliva samples; however they were willing to allow their children to receive a dental examination and the necessary referral forms in order for their child to receive any dental treatment that they may require.

The dmft for the 6-year-olds in this sample (4.86) was considerably lower than other similar studies which reported dmft scores between 6 and 8.^[10-12] Possible reasons could be that these studies were done in the early 2000 and since then there have been changes in the levels of education and awareness in Saudi Arabia. The DMFT for the 12-year-olds (1.31) was also slightly lower than similar studies conducted in other parts of Saudi Arabia.^[11]

The number of caries free children was 25% and this was similar to other studies that reported a caries free prevalence of between 17 and 25.3%.^[10,13,14] Some of the reasons for this discrepancy could be the different age groups that were used, the different settings and the fact that two of these studies were done more than 10 years ago. Since then, the levels of knowledge regarding oral health have improved and this could account for the improvement of the caries levels.

Table 3: The correlation between caries severity and counts of MS and LB

Variable	Category	Severe (%)	Mild (%)	Healthy (%)	Total
MS scores	High CFUs	18 (75)	89 (72)	49 (56)	156
	Low CFUs	6 (25)	35 (28)	38 (44)	79
	LR=6.3 at 2 df; $P=0.04$				
LB scores	High CFUs	23 (96)	97 (78)	46 (53)	166
	Low CFUs	1 (4)	27 (22)	41 (47)	69
	LR=25.9 at 2 df; $P<0.01$				
Saliva BC	Low BC	3 (11)	17 (63)	7 (26)	27
	Medium BC	15 (12)	64 (53)	42 (35)	121
	High BC	6 (7)	43 (49)	38 (44)	87
$\chi^2=4.35$ at 4 df; $P=0.36$					

LB = *Lactobacillus*, MS = *Mutans streptococci*, BC = *Buffering capacity*, CFU = *Colony forming units*, OR = *Odds ratio*, CI = *Confidence interval*, df = *Degrees of freedom*

Table 4: Predictability of dental caries using combinations of risk factors

	No. of respondents	Odds ratio	P value (CI)
High MS CFUs	156	2.44	0.05 (1.3-4.5)
Low LB CFUs	166	4.45	0.00 (2.3-8.3)
Low BC	27	4.72	0.04 (1.1-20.6)
High MS CFUs+High LB CFUs	141	3.51	0.00 (1.9-6.5)
High MS CFUs+Low BC	23	9.4	0.00 (1.27-70.7)
High LB CFUs+Low BC	22	7.86	0.03 (1.0-59.8)
High MS CFUs+High LB CFUs+Low BC	21	8.5	0.02 (1.1-64.2)

High scores ≥10,000 CFUs, LB = *Lactobacillus*, MS = *Mutans streptococci*, BC = *Buffering capacity*, CFU = *Colony forming unit*, CI = *Confidence interval*

There were many more students that were diagnosed with high CFUs compared to low CFUs. This could also be due to poor oral hygiene and a diet rich in fermentable carbohydrates and acidic fizzy drinks that other Saudi studies using a similar population have reported.^[11,14] There was a significant relationship between a high dmft and high counts of LB; confirming results obtained by Gábris *et al.*^[15] A possible reason could be that LB is associated with acid production and the progression of dental caries and as the WHO criteria were used to diagnose dental caries, only frank dental caries was recorded. MS is associated with the initiation of dental caries and it is therefore possible that in early lesions there may be more MS than LB, but in this sample with cavitated and progressed caries, it was expected to find high LB counts. Nishikawara *et al.*,^[5] showed similar results and concluded that LB could be a useful tool for detecting dental caries while MS could not be confidently used in the prediction of caries. Another reason could be that studies have shown that MS is generally found in deep cavities,^[16] while LB is found within the oral cavity including the oral mucosa and saliva.^[17] Therefore by taking saliva it is possible to detect more LB compared to MS even though there may be higher numbers of MS in the oral cavity. Other studies have reported that high numbers of LB have been found in the saliva of children who consume large amounts of simple carbohydrates.^[18,19] Numerous studies done in Saudi Arabia have shown that young children in Saudi Arabia tend to consume large amounts of fizzy drinks and fermentable carbohydrates and this could explain the high amounts of LB that was found in this sample.^[11,20]

There were significant correlations between those with caries and high counts of both MS and LB. The odds of those with high counts of LB and MS were four and two times higher for developing dental caries compared to those with low counts, respectively. This has far reaching implications for the planning of services; if patients with high counts of both LB and MS can be detected early, it is possible to prevent dental caries and save the patient and state resources.

Although the majority of the sample had a high or medium saliva BC, those who had a low saliva BC had statistically more severe caries compared to the rest. These children had on an average 8 or more teeth decayed and this form of rampant caries results in pain, early extractions, and possibly orthodontic treatment in the future. Hence, it is imperative to try and prevent these complications by early detection and intervention. Saliva stimulants and fluoride gels could be administered on a regular basis to ensure that these high risk groups

can be prevented from developing dental caries. These results were similar to other studies which showed a strong correlation between the buffering capacity and the prevalence of dental caries.^[6,21]

When calculating the OR for the correlation between single and multiple combinations of the risk factors and the presence of dental caries, all tests proved to be statistically significant. The highest OR was for combined high MS score and low saliva BC category. This was followed by participants who had all three risk factors present (high MS, high LB, and low saliva BC). This was not unexpected as all of these are risk factors for dental caries and if a patient presents with all three of them, they should be vulnerable to dental caries.

Although the ideal way to predict dental caries using these risk factors would be using a prospective cohort study; due to financial, ethical, and time constraints; this was not possible. The alternative was to perform a cross-sectional study which correlated the risk factors to the presence of dental caries. Therefore, the results from our study could still be used to draw generalized conclusions which included the following; those with high bacteria counts should be detected early and prevention programs should be implemented so that the effects of these bacteria could be reduced. Routine bacterial counts should be a part of the annual dental examination so that patients could be advised as to the oral hygiene and diet practices using these bacterial counts as a guide. Lastly, although saliva BC was not as strongly associated with dental caries on its own, saliva as part of bacterial counts could be a useful predictor of dental caries and should not be overlooked.

CONCLUSION

Although the prevalence of dental caries amongst the 6-year-olds was high, it was considerably lower than results obtained from similar studies in Saudi Arabia and this could be attributed to the improvement of knowledge and access to services. There were more respondents with high CFUs of both bacteria and this could be a cause for the relatively high levels of caries. The majority of respondents had high or medium saliva BC, but amongst the few that had a low BC, almost all of them had rampant caries. There was a strong correlation between high MS, LB, and low BC and the high prevalence of dental caries. The presence of high bacteria counts and low saliva BC can be confidently used to predict dental caries in young children in this population.

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