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Role of Salivary Biomarkers in Caries Risk Assessment

Research Article

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Abstract

Objective: This study was performed to quantify the association of salivary alpha-defensins HNP1 to -3 with caries activity in children and identify salivary defenses that may reduce or stop dental caries development.
Methods: At patients' convenience, unstimulated whole saliva was collected from patients visiting the dental teaching hospital. Laboratory investigations were performed to evaluate salivary PH, α-defensin, total antioxidant, total protein, and cariogenic microorganisms' levels. After that, participants' dental records were reviewed for dental caries activity.
Results: The results showed a significant association between caries activity and different salivary biomarkers (salivary pH, α-defensin, streptococcus mutans, and lactobacilli). For every one-unit increase in salivary pH, the odds of having active dental caries decreased by 89% (OR=0.11, P=0.018), and for every one-unit increase in α-defensin the odds of having active dental caries decreased by 25% (OR=0.75, P=0.004), and the odds of having active dental caries for the participant with salivary streptococcus mutans or lactobacilli levels more than or equal to 105 cfu/ml were 8.8 (OR=8.8, P=0.001) and 22 (OR=22, p≤0.001) times higher than those with levels less than 105 cfu/ml respectively. On the other hand, there were no significant associations between salivary total protein, total antioxidant, and caries activity. However, for every one-unit increase in salivary total protein, the odds of having active dental caries increased by 211% (OR=2.11, P=0.072).
Conclusion: The levels of α-defensin, salivary pH, and cariogenic bacteria can be used as risk assessment markers for screening and assessing caries susceptibility.

Keywords: Risk Assessment; Dental Caries; Prevention; Preventive Dentistry; Salivary Biomarkers.

Introduction

Dental caries is one of the most common chronic childhood problems worldwide. Evidence indicates that it is a result of bacterial infection,[1]; however, it is also modified by several factors, including the host (saliva and teeth) and oral microflora (plaque and bacteria).[2, 3] Salivary defense systems (physiochemical characteristics) are crucial in preventing dental caries such as buffering capacity, pH, varying protein concentrations, salivary flow rate, and secretion of antimicrobial peptides (AMPs).[4]

The most outstanding AMPs are the defensins. Depending on the form of cysteine combination, two types of defensins are documented, namely the a and b defensins.[5] The defensins HNP1 to -3 have been expressed in gingival crevicular fluid, are identified in neutrophils, and have a role in nonoxidative bacterial death. This form of secretion suggests that the defensins may prevent dental caries and protect oral mucosa.[6, 7] Additionally, the defensins have broad antimicrobial action against gram-positive and gram-negative bacteria.[8] Therefore, the expression of α-defensins in saliva may create an extra layer of protection by providing a natural antibiotic barrier.

The normal concentration for salivary HNP1-3 in a healthy individual ranges from being undetectable to ~12μg/ml, HNP1–3 elevated with oral inflammation and loose or exfoliating teeth.[9, 10] However, there is no consensus about the relationship between salivary HNP1-3 levels and dental caries. Some researchers reported that salivary HNP1-3 levels had a protective effect against dental caries.[11-14] On the other hand, Toomarian et al. reported no association between salivary HNP1-3 and dental caries.[15] Also, in 2109, Devarajan and Somasundaram, via

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a systematic review, reported insufficient evidence regarding the relationship between HNP1-3 and dental caries.[5]

The present study aims to quantify the association of salivary a defensins HNP1 to -3 with caries activity in children and identify salivary defenses that may reduce or stop dental caries development.

Materials & Methods

Study Design

The present study was a cross-sectional study where participants had a clinical dental examination to determine caries activity and then provided a saliva sample to measure different salivary components.

Setting

The present study was carried out at the dental teaching hospital of Umm Al-Qura University in Makkah city, Saudi Arabia. Subjects visiting the dental teaching hospital participated at their convenience. However, individuals with medical conditions or individuals who were utilizing any drugs or mouthwashes during the most recent two months before participation in the study were excluded.

Data Sources and Measurements

Dental Clinical examination: During the registration process, each patient was given a complete dental exam by a qualified dentist using a dental mirror, explorer, and orthopantomogram (OPG) radiographs at the dental teaching hospital. Bitewing radiographs were taken whenever needed to overcome any uncertainties. Examination of all teeth and surfaces for dental decay was done. The investigator analyzed and reviewed 250 patients' dental health records to get the caries activity.

Saliva samples: Participants were told not to eat for one hour before the examination. Two ml of unstimulated saliva were collected from every participant by asking him\her to allow passive saliva flow into a clean container. The collected sample was used to determine the total protein, total antioxidant, antimicrobial peptide (α-defensin 1-3), PH, S. Mutans, and lactobacilli levels.

Salivary pH: The salivary pH values were digitally recorded using a pH meter (HORIBA Ltd, Japan), following the manufacturer's instructions.

Salivary S. mutans and lactobacilli levels assessment: The concentration of S. mutans and lactobacilli was determined using Caries Risk Test kits (CRT bacteria, Vivadent, Schaan Liechtenstein, Germany) following the manufacturer's instructions.

Storage of saliva samples: 5 µl of the Nonidet P40 was added to each saliva sample to obtain the final concentration of 0.1%. Vials which contain the saliva samples were tightly sealed and stored at – 80 Co for later analysis.[16]

Protein and antioxidant assessment: Levels of salivary total protein were examined using an auto-analyzer (Technicon RAXT, USA) as indicated by the Biuret strategy.[17] Levels of total antioxidant were dictated by the response of antioxidants in the saliva sample with a characterized measure of hydrogen peroxide as indicated by producer's guidelines (Biodiagnostic, Dokki, Giza, Egypt).

Antimicrobial peptide (α-defensin) assessment: Following manufacture's guidelines (Hycult Biotechnology, Uden, The Netherland), the concentration of HNP (1-3) was evaluated using ELISA kits.

Bias

Non-response could be an issue influencing the findings of a cross-sectional study with a convenience sample and lead to a bias of the measures of the outcome. This may be a critical issue or bias when the participants differ from those who didn't participate.

Sample Size Calculation

Logistic regression of a binary response variable (caries-free) on a continuous variable (α-defensin) with a sample size of 167 observations achieves 90% power at a 0.05 significance level to detect a change in probability of having dental caries by 10% at the mean of α-defensin when α-defensin is increased to one standard deviation above the mean. This change corresponds to an odds ratio of 3.353. An adjustment was made since a multiple regression of the independent variable of interest on the other independent variables in the logistic regression obtained an R-Squared of 0.100.[18]

Statistical Analysis

Descriptive characteristics are reported as means, standard deviations for continuous variables, and numbers and percentages for categorical variables. Categorical variables were tested using chi-square statistics for bivariate analyses, while continuous variables were tested using a two-sample t-test with equal variances. For multivariable analysis, a model was developed using multivariable logistic regression to predict the odds of having active dental caries and inspect the concurrent association of salivary biomarkers on caries activity. The model was adjusted for age and gender. To determine what variables to include, since the goal was exploratory in nature, a backward stepwise logistic regression was used with a probability of removal of 0.2 and a probability of entry of 0.15. All statistics were performed in STATA software (Version 14.2; Stata, college station, TX.). All p-values were two-tailed and interpreted at 0.05 significance level.

Ethical Considerations

After obtaining the ethical approval from the Research Ethics Committee, written informed consents were obtained from the participants after understanding the aim of the study. The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Results

Of the 250 participants, 45 were caries-free (18%). The overall

mean age was 9.3 years (SD +/- 2.3). The mean age for caries-free children was 9.9 years (SD +/- 2.1), while it was 9.1 years (SD +/- 2.3) for children with active caries. Males composed 62% of the participants. Descriptive statistics are summarized in Table 1 for continuous variables and summarized in Table 2 for categorical variables.

On the bivariate level, salivary flow rate, salivary PH, Salivary α-defensin, salivary S. mutans levels, and salivary lactobacilli levels differed significantly among caries-free children when compared to children with dental caries (Table 3).

The multivariable findings are summarized in Table 4. The fitted model shows that, while adjusting for age and gender; and holding salivary PH, salivary total protein, salivary S. mutans, and lactobacilli levels at fixed values, for every one-unit increase in α-defensin, the odds of having active dental caries decreased by 25% (OR = 0.75, P = 0.004).

The fitted model shows that, while adjusting for age and gender; and holding α-defensin, salivary total protein, salivary S. mutans, and lactobacilli levels at fixed values, for every one-unit increase in salivary pH, the odds of having active dental caries decreased by 89% (OR = 0.11, P = 0.018).

The fitted model shows that, while adjusting for age and gender; and holding α-defensin, salivary pH, salivary total protein, and lactobacilli levels at fixed values, the odds of having active dental caries for children with salivary S. mutans levels higher than or equal to 105 cfu/ml were 8.8 times higher than children with salivary S. mutans levels less than 105 cfu/ml (OR = 8.8, P = 0.001).

The fitted model shows that, while adjusting for age and gender; and holding α-defensin, salivary pH, salivary pH, salivary total protein, and S. mutans levels at fixed values, the odds of having active dental caries for children with salivary lactobacilli levels higher than or equal to 105 cfu/ml were 22 times higher than children with salivary lactobacilli levels less than 105 cfu/ml (OR = 22, p≤0.001).

Figure 1 shows the visual representation of the probability of having dental decay as a function of α-defensin and stratified by S. mutans levels. Furthermore, figure 2 shows the visual representation of the probability of having dental decay as a function of α-defensinand stratified by lactobacilli levels.

Discussion

Saliva sampling is not an invasive method to indicate different diseases and contain different microorganisms along with host biological elements, which can be utilized for caries risk appraisal. Presently, managing dental caries is directed toward comprehensive dentistry, including preventive and minimally invasive approaches. Caries risk assessment allows for early detection of risk factors behind the dental caries process, the estimation of caries incidence, and the likelihood of the changes in the activity of carious decays.[19]

The current results indicated that salivary pH could be used as a caries risk assessment indicator. The odds of having active dental caries decreased significantly with the increased pH (Table 1). These results confirm the results of other studies [20, 21] that reported that caries-active individuals had higher amounts and

Table 1. Continuous descriptive statistics.

Variable	Active Caries				Overall	
	No		Yes			
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Age	9.9	2.1	9.1	2.3	9.3	2.3
Salivary flow rate	0.4	0.1	0.3	0.1	0.3	0.1
PH	7.3	0.4	6.9	0.3	7	0.3
Salivary total protein	0.8	0.7	1	0.7	0.9	0.7
Salivary total antioxidant	1.4	0.7	1.5	0.8	1.5	0.8
α-defensin	5.8	4	2.8	1.8	3.3	2.6

Table 2. Categorical descriptive statistics.

Variable	Active Caries				Overall	
	No		Yes			
	No.	%	No.	%	No.	%
Salivary streptococcus mutanslevels (>10^5)						
No	40	88.9	41	20	81	32.40%
Yes	5	11.1	164	80	169	67.60%
Salivary lactobacilli levels (>10^5)						
No	39	86.7	26	12.7	65	26%
Yes	6	13.3	179	87.3	185	74%
Gender						
Female	20	44.4	75	36.6	95	38%
Male	25	55.6	130	63.4	155	62%
Total	45	18%	205	82%	250	100%

Table 3. Bivariate Results.

Variable	Active Caries				P-value
	No		Yes		
Salivary streptococcus mutanslevels (>10^5)					
No [number and percentage]	40	16%	41	16.40%	< 0.0001
Yes [number and percentage]	5	2%	164	65.60%	
Salivary lactobacilli levels (>105)					
No [number and percentage]	39	15.60%	26	10.40%	< 0.0001
Yes [number and percentage]	6	2.40%	179	71.60%	
Salivary flow rate [mean and (S.D.)]	0.36	+/- 0.02	0.31	+/- 0.01	0.001
PH [mean and (S.D.)]	7.3	+/- 0.06	6.9	+/- 0.02	< 0.0001
α-defensin [mean and (S.D.)]	5.8	+/- 0.6	2.8	+/- 0.17	< 0.0001
Note: Categorical variables tested using chi square statistics while continuous variables tested using two-sample t-test with equal variances.					

Table 4. Results from multivariable logistic regression with active dental caries as dependent variable adjusted for age and gender.

	OR	S. E.	P-value	95% C.I
α-defensin	0.75	0.07	0.004	(0.62 - 0.91)
Salivary pH	0.11	0.1	0.018	(0.02 - 0.68)
Salivary total protein	2.11	0.88	0.072	(0.93 - 4.78)
Salivary *streptococcus mutans* levels (≤10^5 vs >10^5)	8.8	5.69	0.001	(2.5 - 31.24)
Salivary *lactobacilli* levels (≤10^5 vs >10^5)	21.85	14.24	< 0.000	(6.09 - 78.41)

Figure 1. Preicted Probability of Having Active Dental Caries by *S.mutans* Levels.

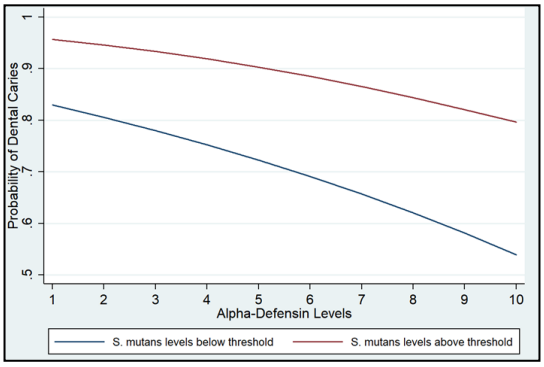
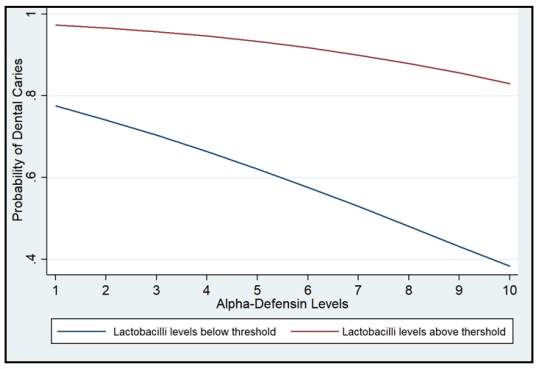


Figure 2. Preicted Probability of Having Active Dental Caries by *Lactobacilli* Levels.



faster acid production rates than caries-free individuals.[20]Also, the mean levels of salivary pH were significantly decreased in caries-active children compared to caries-free controls.[21]

The present study results showed that for every one-unit increase in α-defensin, the odds of having active dental caries decreased significantly (Table 1). These results confirm the previous results, which indicated an inverse correlation between salivary α-defensin and dental caries.[10, 11, 22]

The present study confirms the role of cariogenic microorganisms (*S. mutans and lactobacilli*) in the dental caries process, suggesting more dental caries in participants with more than 105 cfu/ml of cariogenic microorganisms (Table 1). These findings confirmed the results of the previous studies.[23-26] The risk assessment prediction is stronger for lactobacilli as participants with salivary lactobacilli levels 105 cfu/ml or more were 22 times higher than children with salivary lactobacilli levels lower than 10^5

cfu/ml (OR=22 for *lactobacilli* compared to 8.8 for S. mutans). These results demonstrated that appropriate microbiological test systems could compromise a ground work for improving the clinical dental caries risk assessment. [27]

In comparison between caries affected compared to caries-free participants, the salivary total protein concentration was higher in caries-affected individuals. For every one-unit increase in salivary total protein, the odds of having active dental caries increased by 211% (OR = 2.11, P = 0.072). This increase in odd ratio was not significant and disagreed with previous results. The present results confirm the findings of other studies, which showed no consistent relationship between salivary proteins and dental caries.[28, 29] The lack of association between salivary proteins and dental caries may be due to different protein levels with different structures and functions.[15]

In this study, total antioxidant concentration was not associated with dental caries, and these results were similar to the results of other studies.[30, 31] The present results were agreed with that of Ahmadi-Motamayel et al [32], who reported that Salivary and serum TAC levels in caries affected and caries-free groups did not show any significant differences.

Conclusion

In conclusion, α-defensin, salivary pH, and concentration of cariogenic bacteria, especially lactobacilli, can be used as a caries risk assessment marker. These results suggest new tools used for screening and assessing caries vulnerability.

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