Original Article

Relationship between Physicochemical Properties of Saliva and Dental Caries and Periodontal Status among Female Teachers Living in Central Iran

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ABSTRACT

Objectives: There are inconsistent data about the association between saliva properties, dental caries, and periodontal status. In this study, we tried to examine the association between dental caries and periodontal status with salivary viscosity, flow rate, pH, and buffering capacity in adults.

Methods: In the present cross-sectional study, 450 female teachers were randomly selected from schools located in Yazd, Iran. Oral examinations were conducted, and unstimulated saliva samples were collected. Salivary viscosity, flow rate, pH, and buffering capacity were assessed. The salivary physicochemical properties were compared among teachers with different types of oral health. Analyses were done using the Statistical Package for the Social Sciences version 16.

Results: In total, 431 female teachers aged 40.45 ± 8.18 years were included in the study. Salivary flow rate, buffering capacity, pH, and viscosity, community periodontal index status were not significantly different in participants with and without tooth caries. There was a reverse linear association between salivary pH and flow rate with the decayed, missed, and filled teeth index ($P < 0.05$). The saliva buffering capacity was not significantly related to dental properties. Those with bleeding on probing had lower salivary pH, and buffering capacity compared to those with healthy gum. However, the salivary resting flow rate was not different in participants with bleeding on probing and healthy participants.

Conclusion: Based on our results, saliva properties might be important predictors in oral health status. This means that any change in saliva combination might affect periodontal and dental diseases. Future prospective studies are recommended to confirm these results.

KEYWORDS: *Dental caries, periodontal index, physicochemical properties, saliva*

INTRODUCTION

Saliva is a body fluid secreted by salivary glands which essentially contains water, proteins, glycoproteins, electrolytes, small organic molecules, and compounds that is transported from the bloodstream.[1,2] It plays an important role in maintaining oral health due to its biological functions such as food swallowing, lubrication of oral tissues, and washing off food debris.[3] Saliva also helps maintaining the integrity of dental tissues and especially helps in prevention of dental caries.[2] Furthermore, it has three specific roles in the oral cavity. First, it protects the enamel against demineralization caused by the acids. Second, it facilitates the remineralization of incipient caries and then the last one it has some antimicrobial functions.[3,4]

Saliva can be assessed by some characteristics, such as flow rate, buffering capacity, hydrogen-ion

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Access this article online

Quick Response Code:

Website: www.jispcd.org

DOI: 10.4103/jispcd.JISPCD_391_17

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concentration (pH), and its consistency.[3,5-9] Alteration in any of these characters may increase the risk of caries.[10] Saliva can buffer acids, and this ability is essential for maintaining pH values in the oral cavity. Bicarbonate ions play an important role in determining the pH and buffering capacity of saliva that can help protect teeth against attack from acids which are produced by bacteria.[11] Dental caries is probably the most common consequence of lacking saliva.[12]

The reduction in salivary flow rate may cause severe caries and mucosal inflammations.[13,14] Individuals with impaired saliva flow rate often show higher risk of caries or the susceptibility to have caries.[15,16] However, the studies were inconsistent in their results particularly in those with normal flow rate.[2] Moreover, an increase in the antioxidant activity of the saliva may lead to the increase in the suspension of proteins and cariogenic activity.[17] Some documents claim that buffering capacity of saliva can modify the erosive process in teeth.[12,18] Saliva also can affect periodontal status.[19-21] In a study about salivary antioxidant capacity, it was found that women had significantly lower total antioxidant status compared to men, regardless of periodontal health. Periodontal damage was also associated with reduced salivary antioxidant status.[22] In addition, inflammatory processes in the mouth, such as periodontal disease, have been related to the decrease of saliva antioxidants.[21] Although challenges remain ahead, saliva fluid is useful for future application to diagnose periodontal diseases and to prognosticate periodontal treatment outcomes.[20,23] As studies examined the association between saliva properties and dental caries and periodontal status were conflicting and a limited number of studies have been conducted investigating such association in the Middle East, in the present study, we tried to explore such association in a large sample of female adults controlling the maximum number of possible confounders.

MATERIALS AND METHODS

STUDY DESIGN AND SAMPLE SIZE
This was a cross-sectional study that was conducted from September 2016 to March 2017 on 20-to 60-year-old female teachers working in schools of the city of Yazd in Central Iran. This study was in the context of a larger cross-sectional study which is explained somewhere else.[24] The permission was obtained from the Ethics Committee of Shiraz University of Medical Sciences and the Yazd province education organization. The study protocol for the present analysis was approved by the Research Council of Shiraz University of Medical Sciences, and all participants filled an informed consent before entering the study (approval no: 94-01-03-9223).

Randomized cluster sampling method was used in the study, and 450 teachers were invited. The minimum required sample size was calculated to be 151 according to the mean decayed, missed, and filled teeth (DMFT) of adults in a similar study,[25] the accuracy of 1, and α = 0.05. However, as the cluster randomization was used for sampling, we had enough resources and the study had objectives other than the present study we tried to include 450 participants.

The inclusion criteria were to be a female teacher of chosen schools, and then, participants with diabetes mellitus and pregnant women were omitted. Information on general characteristics as well as education, economic status, physical activity, dietary food and supplement intake, and self-assessment of oral hygiene condition were obtained using self-administered questionnaires. The anthropometric measurements were conducted by a trained nutritionist, and all participants were then visited by a general dentist for assessment of dental caries to observe their dental status and gingival status. After that collecting, the saliva sample was done at their schools.

ANTHROPOMETRIC MEASUREMENTS
In all cases, anthropometric parameters (height, weight, waist circumference, and hip circumference) were measured by a nutritionist. Weight was measured using a digital scale (SECA, model 813) with an accuracy of 100 g while the participant had minimum cloths on. Height was measured using a tape measure mounted on the wall with an accuracy of 0.5 cm while the participants were in the standing position, and body mass index (BMI) was calculated by dividing weight (in kg) by height squared (in square meters).

ORAL EXAMINATION
The oral examination was carried out inside of each school by one general dentist to minimize the between assessor variabilities. The dentist was trained for all the examinations done in the present study. Teachers sat on a chair, and their teeth and gingiva were examined by means of a headlight and disposable dental mirror, dental probes, and tongue blades. Samples' DMFT and community periodontal index (CPI) were assessed using the WHO criteria.[26]

COLLECTING SALIVA SAMPLES
Teachers were told to be present at school between 8:00 and 10:00 in the morning to collect their saliva. They were asked to brush their teeth without any saliva stimulator, and they were fast from 2 h before collecting the samples.[27] Teachers were asked to hold their head down, not swallow, and split all their saliva in a collecting cap for 10 min. Their salivary glands should not be stimulated by any stimulator to collect resting saliva.

SALIVA VISCOSITY

The consistency of saliva was obtained by visual examination. Healthy saliva is watery and clear. In a case with low production rate of saliva, it becomes frothy, stringy, bubbly, or very sticky.[28] Saliva samples were categorized to normal (watery) and high viscosity (sticky or bubbly) groups based on this visual examination.

SALIVA FLOW RATE

The collected saliva in caps was weighted by a digital scale (model: Precision Balance M.T Electronic Balance K-500BH, 0.01–500 g scale, made in Hong Kong) before and after saliva collection.

SALIVARY pH AND BUFFERING CAPACITY

Salivary pH was checked right after collecting saliva using a pH meter at school. After that, 1 ml of 0.1 N hydrochloric acid (HCL) was added to 1 ml of saliva for calculating the buffering capacity according to Ericsson method.[17] For assessing an accurate range of pH, a digital pH meter which shows up to two decimal places was used (model no: AZ8686 made in Taiwan).

ASSESSMENT OF COVARIATES

Other variables were also collected by administration of self-administered questionnaires. The following data were collected: age (in years), marital status (single/married), participants' education (college/bachelor's degree/master's degree or higher), menstruation status (yes/no), oral contraceptive use (yes/no), history of cardiovascular diseases, history of type 2 diabetes, cardiovascular diseases, metabolic syndrome or other chronic diseases (yes/no), family history of diabetes (yes/no), and Vitamin D or multivitamin-mineral supplement use (yes/no). Economic status was assessed using a 9-item self-administered questionnaire. In the questionnaire, we asked about the number of family members, husband's occupation, the head of household (husband/herself/other family members), house ownership (owner/tenant), house type (apartment/house), number of bedrooms in the house, car ownership (yes/no), number of cars owned by the family, and family income per month. Data on physical activity were obtained using the international physical activity questionnaire (short format).[29] The information gathered from this questionnaire was converted to metabolic equivalent hours per week and participants were placed in two categories (sedentary and active). The usual toothbrushing style (lower than once a day/once a day/twice a day/more than twice a day) was also asked in the questionnaire.

STATISTICAL ANALYSIS

The normal distribution of quantitative data was checked using Kolmogorov–Smirnov status.

The comparison of quantitative and qualitative variables between participants with and without tooth decay was examined using independent samples Student's *t* and Chi-square tests, respectively.

The linear association of saliva pH, flow rate, and buffering capacity with number of decayed (D), missed (M), and filed (F) teeth and also the DMFT index was assessed using the linear regression in crude and two multivariable-adjusted models. Age was adjusted in the first model, and other possible effect modifiers including BMI, physical activity level (sedentary/active), menstruation status (yes/no), education (college/bachelor's degree/master's degree), marriage status (single/married), economic status (low/middle/high), oral contraceptive use (yes/no), history of chronic diseases (yes/no), and toothbrushing (lower than once a day/once a day/twice a day/more than twice a day) were additionally adjusted in the second model.

The comparison of salivary pH, buffering capacity, and flow rate across periodontal status was assessed using analysis of covariance with Bonferroni correction in the same multivariable-adjusted models. The statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS, version 16.0 for Windows, 2006, SPSS, Inc., Chicago, IL, USA). $P < 0.05$ was considered as statistically significant.

RESULTS

In total, 431 participants (95.8%) aged 40.45 ± 8.18 years had complete data to be entered to be included in the current analysis; of these, 163 participants (37.8%) did not have any decayed tooth and 268 participants (62.2%) were diagnosed with at least one decayed tooth. Participants with at least one decayed tooth were younger ($P = 0.007$) and had a lower number of missed teeth ($P = 0.004$) compared to those without any decayed tooth. Furthermore, a lower proportion of this group experienced menstruation ($P = 0.038$). This is while the number of DMF teeth was significantly higher ($P < 0.001$) in participants with decayed teeth compared to those without any decayed teeth. Participants with and without decayed teeth were not significantly different according to BMI, filled teeth, salivary flow rate, buffering capacity, pH and viscosity, CPI status, marriage status, education status, economic status, OCP use, physical activity, disease history, and toothbrushing habit [Table 1].

The linear association between salivary physic-chemical properties and number of DMFT as well as the DMFT

Table 1: General characteristics of study participants[a]				
	Dental decay status		Total population	*P*
	Participants without dental decay (*n*=163)	Participants with dental decay (*n*=268)		
Age (year)	41.80±7.74	39.63±8.35	40.45±8.18	0.007
BMI (kg/m²)	27.94±4.76	27.71±4.61	27.80±4.66	0.625
Missed teeth	5.61±5.21	4.22±4.11	4.74±4.60	0.004
Filled teeth	7.37±4.84	6.98±5.20	7.13±5.06	0.431
DMFT index	12.99±6.74	15.86±7.08	14.77±7.09	<0.001
Saliva flow rate (ml/min)	0.72±0.37	0.69±0.41	0.70±0.39	0.378
Saliva buffering capacity (pH reduction)	4.22±1.20	4.33±1.21	4.29±1.20	0.373
Saliva pH	6.07±0.91	5.99±1.04	6.01±0.99	0.352
Saliva viscosity (%)				
Normal	89.0	91.0	90.3	0.505
Low	11.0	9.0	9.7	
CPI status				
Healthy	19.6	23.0	21.7	0.147
Bleeding on probing	18.4	26.2	23.1	
Pocket depth of 3-5 mm	11.0	8.7	9.6	
Bleeding on probing and calculus	50.9	42.1	45.5	
Marriage status (%)				
Single	7.4	10.1	9.1	0.390
Married	92.6	89.9	90.9	
Education (%)				
College	20.4	18.4	19.1	0.161
Bachelor's degree	64.8	72.2	69.5	
Master's degree or higher	14.8	9.4	11.4	
Economic status (%)				
Low	31.3	31.1	31.2	0.995
Medium	32.5	33.0	32.8	
High	36.2	36.0	36	
Menstruation (%)				
Yes	20.2	12.3	15.3	0.038
No	79.8	87.7	84.7	
OCP use (%)				
Yes	3.1	7.9	6.1	0.059
No	96.9	92.1	93.9	
Physical activity (%)				
Sedentary	79.0	73.3	75.5	0.202
Active	21.0	26.7	24.5	
Disease history (%)				
Yes	49.1	39.2	42.9	0.046
No	50.9	60.8	57.1	
Toothbrushing (%)				
Lower than once a day	9.9	10.2	10.1	0.735
Once a day	51.2	50.9	51.0	
Twice a day	31.5	34.0	33.0	
More than twice a day	7.4	4.9	5.9	

[a]Values are represented as mean±SD, otherwise indicated. CPI=Community periodontal index, OCP=Oral contraceptives, SD=Standard deviation, BMI=Body mass index, DMFT=Decayed, missed, and filled teeth

index is represented in Table 2. The analyses revealed that there is a linear reverse association between salivary pH and the number of decayed teeth ($\beta = -0.456$, $P = 0.016$), filled teeth ($\beta = -0.488$, $P = 0.048$), and DMFT index ($\beta = -1.064$, $P = 0.002$) in the crude analysis. These associations remained significant after adjustment for age in the first model (decayed teeth, $\beta = -0.419$, $P = 0.026$; filled teeth, $\beta = -0.517$, $P = 0.037$; and DMFT index, $\beta = -1.081$, $P = 0.002$). However, the association was only remained significant for DMFT

Table 2: The linear association between saliva parameters and the number of decayed, missed, and filled teeth and the decayed, missed, and filled teeth index in crude and multivariable-adjusted models[a]

Saliva parameters	Decayed teeth			Missed teeth			Filled teeth			DMFT index		
	r^2	β	*P*	r^2	β	*P*	r^2	β	*P*	r^2	β	*P*
pH												
Crude	0.013	−0.456	0.016	0.001	−0.110	0.625	0.009	−0.488	0.048	0.022	−1.064	0.002
Model 1[b]	0.029	−0.419	0.026	0.007	−0.137	0.543	0.015	−0.517	0.037	0.023	−1.081	0.002
Model 2[c]	0.049	−0.380	0.052	0.023	−0.108	0.645	0.034	−0.485	0.058	0.028	−0.977	0.006
Buffering capacity												
Crude	0.001	0.099	0.526	0.005	−0.270	0.143	0.006	−0.321	0.113	0.008	−0.534	0.059
Model 1[b]	0.018	0.098	0.527	0.011	−0.269	0.143	0.011	−0.321	0.113	0.009	−0.534	0.060
Model 2[c]	0.042	0.132	0.412	0.029	−0.290	0.130	0.032	−0.324	0.122	0.017	−0.515	0.080
Resting flow rate												
Crude	0.002	−0.396	0.405	0.004	−0.695	0.216	0.011	−1.343	0.030	0.019	−2.454	0.004
Model 1[b]	0.018	−0.270	0.569	0.010	−0.790	0.161	0.017	−1.445	0.020	0.020	−2.515	0.004
Model 2[c]	0.040	−0.166	0.736	0.028	−0.826	0.160	0.039	−1.473	0.022	0.028	−2.460	0.006

[a]All analyses were conducted using the linear regression model, [b]Adjusted for age, [c]Adjusted for variables in model one plus BMI, physical activity level (sedentary/active), menstruation status (yes/no), education (college/bachelor's degree/master's degree), marriage status (single/married), economic status (low/middle/high), oral contraceptive use (yes/no), history of chronic diseases (yes/no), and toothbrushing (lower than once a day/once a day/twice a day/more than twice a day). BMI=Body mass index, DMFT=Decayed, missed, filled teeth

index after further adjustment for BMI, physical activity level (sedentary/active), menstruation status (yes/no), education (college/bachelor's degree/master's degree), marriage status (single/married), economic status (low/middle/high), oral contraceptive use (yes/no), history of chronic diseases (yes/no), and toothbrushing (lower than once a day/once a day/twice a day/more than twice a day) in the second model (β = −0.977, *P* = 0.006). The salivary flow rate was also inversely associated with the number of missed teeth and the DMFT index either in crude (filled teeth, β = −1.343, *P* = 0.030 and DMFT index, β = −2.454, *P* = 0.004) and fully adjusted models (filled teeth, β = −1.473, *P* = 0.022 and DMFT index, β = −2.460, *P* = 0.006). The saliva buffering capacity was associated with all dental properties neither in crude nor in multivariable-adjusted models [Table 2].

It was revealed that salivary pH is significantly different based on CPI categories either in crude and multivariable-adjusted models [Table 3]. Between-group comparisons revealed that those with bleeding on probing had a significantly lower salivary pH and buffering capacity (*P* < 0.001) compared to other CPI status groups. Although the salivary resting flow rate was not different in participants with bleeding on probing and healthy participants, those with bleeding on probing had a significantly lower saliva flow rate compared to participants with pocket depth of 3–5 mm and those with either blooding on probing and calculus (*P* < 0.001). The other between-group comparisons of salivary physic-chemical properties according to CPI status are provided in Table 3.

DISCUSSION

The present cross-sectional study on 431 adult female teachers showed that there is a linear inverse association between salivary pH and the DMFT index. We also found that the salivary flow rate is negatively associated with the number of missed teeth and the DMFT index. We also found that salivary pH, flow rate, and buffering capacity are significantly different in participants with different CPI status.

The present study was the first study which tried to examine the association between salivary properties and dental caries and periodontal status after adjusting for the maximum number of potential confounders. Furthermore, we tried to recruit a large number of participants to increase the statistical power of the study. There are also some limitations that should be considered while interpreting our results. Due to the cross-sectional nature of the current project, causality cannot be inferred and prospective observational studies are highly needed to confirm our findings. In addition, although we have tried to control for several confounding variables in our analyses, residual confounding from unknown or unmeasured factors is inevitable. It should also be kept in mind that the present study was a done in a sample of female adults working in schools across the Yazd city; therefore, generalizing our findings to the Iranian adults must be considered with caution.

While the published evidence is scarce, the association between saliva physicochemical properties and dental caries had been explored in a number of studies, particularly among children.[30-34] For instance, a study which compared the salivary properties in

Table 3: Mean saliva parameters based on the community periodontal index of treatment need community periodontal index in crude and multivariable-adjusted models[1]

Saliva parameters	CPI status				P
	Healthy (*n*=90)	Bleeding on probing (*n*=96)	Pocket depth of 3-5 mm (*n*=40)	Bleeding on probing and calculus (*n*=189)	
pH					
Crude	6.44±0.09[a]	5.37±0.09[b]	6.77±0.14[a]	6.02±0.06[c]	<0.001
Model 1[2]	6.46±0.09[a]	5.38±0.09[b]	6.76±0.14[a]	6.01±5.88[c]	<0.001
Model 2[3]	6.54±0.10[a]	5.38±0.09[b]	6.76±0.15[a]	5.99±0.07[c]	<0.001
Buffering capacity					
Crude	4.57±0.12[a,c]	3.83±0.12[b]	5.01±0.18[c]	4.27±0.08[a]	<0.001
Model 1[2]	4.58±0.12[a,c]	3.83±0.12[b]	5.01±0.18[c]	4.26±0.08[a]	<0.001
Model 2[3]	4.55±0.13[a,c]	3.86±0.12[b]	5.06±0.20[c]	4.25±0.09[a,b]	<0.001
Resting flow rate					
Crude	0.68±0.04[a,b]	0.55±0.04[b]	0.80±0.06[a]	0.76±0.03[a]	<0.001
Model 1[2]	0.69±0.04[a,b]	0.56±0.04[b]	0.79±0.06[a]	0.76±0.03[a]	<0.001
Model 2[3]	0.69±0.04[a,b]	0.56±0.04[b]	0.81±0.06[a]	0.75±0.03[a]	<0.001

[1]Values are shown as mean±SEM. All comparisons were conducted using the analysis of covariance. Values which do not share common superscripts in each row are significantly different ($P<0.05$), [2]Adjusted for age and total energy intake, [3]Adjusted for variables in model one plus BMI, physical activity level (sedentary/active), menstruation status (yes/no), education (college/bachelor's degree/master's degree), marriage status (single/married), economic status (low/middle/high), oral contraceptive use (yes/no), history of chronic diseases (yes/no), and toothbrushing (lower than once a day/once a day/twice a day/more than twice a day). BMI=Body mass index, SEM=Standard error of mean, CPI=Community periodontal index

caries-active and caries-free children revealed that salivary flow and pH were significantly lower in caries-active participants.[35] Furthermore, a study conducted in Medina, Saudi Arabia, showed that there is an association between low buffering capacity and higher prevalence of dental caries among children.[31] In another study published in 2014, Animireddy *et al*. revealed that the mean salivary flow rate, salivary pH, and salivary buffering capacity is significantly higher and the salivary viscosity is significantly lower among caries-free participants.[30] In contrast, data about the association between salivary properties and dental caries are fewer in adults. Conducting a case–control study on participants with ($n = 20$) and without diabetes mellitus ($n = 20$), Seethalakshmi *et al*. showed that there is a significant relationship between the diabetes mellitus and increased incidence of dental caries and periodontitis and there was also a significant reduction in the salivary pH in diabetes mellitus patients, compared to that of nondiabetic participants. Therefore, they concluded that salivary pH might be associated with dental caries in patients with diabetes mellitus. They also found an inverse association between saliva pH and DMFT index.[33] A large-scale study in 1763 American patients showed that salivary pH, flow rate, and buffering capacity are not associated with dental caries in adult patients.[36] This is while we found a reverse association between nonstimulated saliva pH and flow rate and dental caries, but the relationship was not significant for buffering capacity. A number of studies have proposed that resting pH might not be

a predictor for dental decay;[37,38] however, the studies providing such evidence are accumulating.

In the present study, we found that saliva pH and buffering capacity are lowest among participants with bleeding on probing while the saliva pH was highest in those participants who had both bleeding on probing and calculus and the buffering capacity was highest in those with pocket depth of 3–5 mm. A study published recently has shown that participants with bleeding on probing and participants with periodontal pockets >4 mm as well as clinical attachment loss with or without bleeding on probing have a significantly higher saliva pH compared to healthy controls.[39] A higher pH is associated with increased proteolytic activity in microorganisms which favors the deposition of calcium phosphate and therefore the promotion of plaque mineralization. It is also suggested by previous studies that higher salivary flow rate is significantly associated with development of periodontitis.[40] In the present study, we also found a higher saliva unstimulated flow rate in those with pocket depth of 3–5 mm and those with both bleeding on probing and calculus compared to those with just bleeding on probing; however, the difference was not statistically significant compared to healthy participants. Our results are in line with the previous studies. In the present study, we tried to include a large sample of female adults, and this large sample size led us to explore the association after controlling the maximum number of confounding variables. This is while the other studies could not take the other confounders into account.

Conclusion

We found that there is a linear inverse association between salivary pH and the DMFT index. We also found the salivary flow rate is negatively associated with the number of missed teeth and the DMFT index. We also found that saliva pH, flow rate, and buffering capacity are significantly different in participants with different levels of periodontal diseases. Saliva properties are important in oral health. Changes in its physicochemical properties might negatively affect dental and periodontal status. Prospective cohort studies are highly recommended to confirm the cause and effect association for the relationships we found in the current study.

Conflicts of interest

There are no conflicts of interest.

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