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Research Article

Relationship between Salivary Flow Rate, Salivary Protein Concentration and Dental Caries Experience among Young Adults in Peshawar, Pakistan

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ABSTRACT

Background: Dental caries is a multifactorial, biofilm-mediated disease influenced by microbial, dietary, behavioral and host-related factors. Saliva is an important host-protective factor because it contributes to oral clearance, acid buffering, antimicrobial defense and remineralization of dental hard tissues. Both the quantity and composition of saliva may influence susceptibility to dental caries. Salivary flow rate and protein concentration are relatively simple, non-invasive parameters that may provide useful information about an individual's caries experience.

Objective: To determine the relationship between unstimulated salivary flow rate, total salivary protein concentration and dental caries experience among young adults in Peshawar, Pakistan.

Methods: An analytical cross-sectional study was conducted among 150 young adults aged 18–30 years in Peshawar, Pakistan. Dental caries experience was assessed using the Decayed, Missing and Filled Teeth (DMFT) index. Unstimulated whole saliva was collected over five minutes under standardized conditions. Salivary flow rate was calculated in mL/min, while total salivary protein concentration was determined using a colorimetric biochemical assay and expressed in mg/mL. Descriptive statistics, independent-samples t-test, Pearson correlation and multiple linear regression were performed. Statistical significance was set at $p<0.05$.

Results: The mean age of participants was 23.8±3.1 years. The mean DMFT score was 3.2±2.8. The mean unstimulated salivary flow rate was 0.31±0.14 mL/min, while the mean total salivary protein concentration was 1.02±0.36 mg/mL. Participants with high caries experience had significantly lower salivary flow rates than those with low/no caries experience (0.27±0.13 vs. 0.36±0.12 mL/min; $p<0.001$). Conversely, total salivary protein concentration was significantly higher among participants with high caries experience (1.12±0.38 vs. 0.92±0.30 mg/mL; $p=0.002$). Salivary flow rate demonstrated a significant negative correlation with DMFT ($r=-0.36$, $p<0.001$), whereas total protein concentration demonstrated a significant positive correlation with DMFT ($r=0.29$, $p<0.001$). In multivariable regression analysis, salivary flow rate and total protein concentration remained significantly associated with DMFT after adjustment for age, sex, sugar consumption and tooth-brushing frequency.

Conclusion: Reduced unstimulated salivary flow rate and increased total salivary protein concentration were associated with greater dental caries experience among young adults in Peshawar. The findings support the potential value of salivary parameters as supplementary indicators of caries susceptibility. However, longitudinal studies and investigation of individual salivary proteins are required before these parameters can be considered reliable predictive biomarkers.

Keywords: Dental Caries, Saliva, Salivary Flow Rate, Salivary Proteins, DMFT, Young Adults, Peshawar, Caries Biomarkers.

INTRODUCTION

Dental caries remains one of the most common oral diseases worldwide and represents a major public-health concern. It is a multifactorial disease produced through complex interactions between cariogenic microorganisms, fermentable dietary carbohydrates, susceptible tooth surfaces and host protective factors. The disease develops when repeated periods of acid production by dental biofilm result in a sustained imbalance between demineralization and remineralization of dental hard tissues [1,2].

Although microorganisms and dietary carbohydrates are central to caries development, the host environment plays an important modifying role. Among host factors, saliva is particularly important because it continuously bathes the oral tissues and teeth and provides multiple protective functions. These include lubrication, mechanical cleansing, buffering of bacterial acids, antimicrobial activity, maintenance of mineral equilibrium and promotion of enamel remineralization [3,4].

The protective effects of saliva are strongly influenced by its rate of secretion. Adequate salivary flow facilitates the clearance of food particles, sugars, microorganisms and metabolic acids from the oral cavity. Saliva also provides bicarbonate and other buffering components that contribute to recovery of oral pH following carbohydrate exposure. Reduced salivary secretion may consequently increase the duration of acid exposure and compromise the natural protective mechanisms of the oral cavity [3].

Unstimulated saliva is particularly important because basal salivary secretion provides continuous protection in the absence of food-related stimulation. Unstimulated whole saliva originates primarily from the submandibular and sublingual glands, with contributions from other oral sources. Salivary flow varies between individuals and is affected by hydration, circadian rhythm, age, medications, systemic disease, psychological conditions and collection methodology. Standardization of saliva collection is therefore essential for reliable measurement [5].

Saliva is not simply a watery secretion. It contains electrolytes, enzymes, glycoproteins, antimicrobial peptides, immunoglobulins and numerous other proteins involved in oral defense and tissue homeostasis. Salivary proteins contribute to formation of the acquired enamel pellicle, regulate microbial adhesion, participate in mineral homeostasis and provide antimicrobial activity [4].

Total salivary protein concentration represents the combined concentration of multiple salivary proteins. Although it does not provide information about individual proteins, it is relatively inexpensive and technically simple to measure. This makes it potentially useful in studies investigating the relationship between salivary composition and dental caries, particularly in settings where advanced proteomic techniques are not readily available.

The relationship between salivary proteins and dental caries, however, is complex. An earlier systematic review by Martins et al. identified only seven studies meeting its inclusion criteria and concluded that evidence was insufficient to establish salivary proteins as definitive biomarkers of dental caries. Nevertheless, some of the included studies reported associations between dental caries and protein phenotype, total protein concentration and protein molecular weight [6].

More recent evidence has provided stronger support for investigating salivary proteins. Ahmad et al. conducted a systematic review involving 22 studies and 1,551 participants and reported significant differences in several salivary proteins between caries-active and caries-free individuals. Alpha-amylase, acidic proline-rich protein-1, histatin-5, lactoperoxidase and mucin-1 were reported at higher levels among caries-active individuals, whereas carbonic anhydrase 6, proteinase-3 and statherin were higher among caries-free individuals. However, the authors found conflicting evidence regarding immunoglobulin A and total protein concentration [7].

A further systematic review published in 2024 evaluated the diagnostic potential of salivary biomarkers for dental caries. Sixteen studies were included, and all reported significant differences in at least some salivary molecules between participants with and without caries. Proteins were among the most extensively investigated salivary biomarkers, particularly alpha-amylase and mucins. However, methodological heterogeneity and differences in caries assessment limited definitive conclusions regarding diagnostic accuracy [8].

The relationship between salivary flow and protein concentration is also biologically important. Protein concentration is influenced by the amount of water secreted in saliva. A reduction in flow can result in greater protein concentration because of reduced dilution, even if the absolute secretion of proteins does

not increase. Consequently, evaluation of protein concentration without consideration of salivary flow may produce an incomplete interpretation of salivary physiology.

Recent evidence from Pakistan further supports investigation of salivary parameters in relation to dental caries. A large cross-sectional study of 516 Pakistani schoolchildren found that unstimulated salivary flow rate, pH and buffering capacity were significantly higher in caries-free participants than in caries-active participants [9]. Although that investigation involved schoolchildren in Karachi rather than young adults in Peshawar, it provides important evidence that salivary characteristics are associated with caries status within the Pakistani population.

Young adults represent an important population for investigating these relationships. By this age, permanent dentition has been established and cumulative exposure to cariogenic dietary patterns and oral-hygiene practices can be assessed through DMFT. At the same time, young adults are generally less affected by age-related systemic diseases and medications that can substantially alter salivary secretion.

Peshawar is the capital city of Khyber Pakhtunkhwa and an important educational, healthcare and commercial center. The population includes individuals with diverse socioeconomic, educational and behavioral backgrounds. Dietary habits, oral-hygiene practices, dental attendance and access to preventive services may differ from those in other regions of Pakistan. Consequently, locally generated evidence is necessary to understand the biological factors associated with dental caries in this population.

Despite increasing international interest in salivary biomarkers, limited evidence is available concerning the combined relationship between salivary flow rate, total salivary protein concentration and dental caries experience among young adults in Peshawar.

Therefore, the present study was conducted to determine the relationship between unstimulated salivary flow rate, total salivary protein concentration and dental caries experience among young adults in Peshawar, Pakistan.

MATERIALS AND METHODS

Study Design

An analytical cross-sectional study was conducted to investigate the relationship between unstimulated salivary flow rate, total salivary protein concentration and dental caries experience among young adults.

Study Area and Setting

The study was conducted in Peshawar, Khyber Pakhtunkhwa, Pakistan.

Peshawar is the capital and largest major urban center of Khyber Pakhtunkhwa and serves as an important healthcare and educational hub for the province. The city has a diverse population representing different socioeconomic and educational backgrounds.

Participants were recruited from a dental teaching institution in Peshawar. Clinical examination and saliva collection were conducted in the dental clinical facilities, while biochemical analysis of saliva was performed in the associated laboratory.

Peshawar was selected as the study area because of the availability of dental clinical and laboratory facilities and the large young-adult population accessible through dental and educational institutions. The setting also provided an opportunity to generate locally relevant evidence concerning salivary factors associated with dental caries in young adults.

Study Duration

The study was conducted over a period of six months.

Study Population

The study population consisted of young adults aged 18–30 years attending the selected dental institutions in Peshawar.

A total of 150 participants were included in the final analysis.

Inclusion Criteria

Participants were eligible if they:

1. were 18–30 years of age;
2. had permanent dentition;
3. were apparently healthy;
4. provided written informed consent;
5. were able to cooperate with dental examination; and
6. were able to provide an unstimulated saliva sample.

Exclusion Criteria

Participants were excluded if they:

- had diagnosed salivary-gland disease;
- had systemic disease known to substantially affect salivary secretion;
- were taking medications associated with significant xerostomia;
- had received antibiotics within the preceding four weeks;

- had undergone radiotherapy involving the head and neck;
- were undergoing orthodontic treatment; or
- were unable to complete saliva collection.

Clinical Assessment of Dental Caries

Dental caries experience was assessed using the Decayed, Missing and Filled Teeth (DMFT) index.

Each permanent tooth was examined clinically and classified as decayed, missing due to caries or filled. The DMFT score was calculated as:

DMFT = D + M + F

where:

- **D** = number of decayed permanent teeth;
- **M** = number of permanent teeth missing due to caries; and
- **F** = number of filled permanent teeth.

For group comparison, participants were categorized into low/no caries experience and high caries experience based on their DMFT score. DMFT was also analyzed as a continuous variable for correlation and regression analyses.

Saliva Collection

Unstimulated whole saliva was collected between 9:00 am and 12:00 noon to reduce the effect of circadian variation.

Participants were instructed not to eat, drink, smoke, chew gum or brush their teeth for at least one hour before saliva collection.

Participants were seated comfortably with their head slightly tilted forward. Following a short resting period, saliva was allowed to accumulate naturally in the floor of the mouth and was expectorated into a sterile graduated collection tube.

Saliva was collected for five minutes. The total volume was recorded, and unstimulated salivary flow rate was calculated as:

Salivary flow rate (mL/min) = total volume of saliva collected (mL) ÷ collection time (min).

The standardized collection approach was based on established recommendations for saliva collection [5].

Determination of Total Salivary Protein

Following collection, saliva samples were transferred to the laboratory and processed to remove cellular debris and particulate material. Total salivary protein concentration was determined using a standardized colorimetric protein assay. Bovine serum albumin was used as the calibration standard, and absorbance was measured using a spectrophotometer.

Protein concentration was expressed as mg/mL.

Laboratory personnel were blinded to participants' dental caries status during biochemical analysis.

Assessment of Potential Confounders

Information regarding age, sex, frequency of tooth brushing, sugar consumption and consumption of sugar-sweetened beverages was obtained using a structured questionnaire. These variables were considered potential confounding factors because dietary and oral-hygiene behaviors may influence dental caries independently of salivary parameters.

Statistical Analysis

Statistical analysis was performed using SPSS. Continuous variables were expressed as mean±standard deviation, while categorical variables were expressed as frequencies and percentages.

The independent-samples t-test was used to compare salivary flow rate and protein concentration between participants with low/no and high caries experience.

Pearson correlation analysis was used to determine the relationships between:

- salivary flow rate and DMFT;
- total salivary protein concentration and DMFT; and
- salivary flow rate and total protein concentration.

Multiple linear regression was performed with DMFT as the dependent variable. Salivary flow rate and total protein concentration were entered as primary independent variables, while age, sex, sugar consumption and tooth-brushing frequency were included as potential covariates.

A p-value of <0.05 was considered statistically significant.

Ethical Considerations

Ethical approval was obtained from the relevant institutional research ethics committee before commencement of the study. Written informed consent was obtained from all participants. Participants were informed regarding the purpose and procedures of the research and were free to withdraw at any time. Confidentiality of participant information was maintained throughout the study.

RESULTS

Demographic Characteristics

A total of 150 young adults were included in the study. The mean age was 23.8±3.1 years, with an age range of 18–30 years.

Among the participants, 78 (52.0%) were male and 72 (48.0%) were female.
The overall mean DMFT score was 3.2±2.8.
The mean unstimulated salivary flow rate was 0.31±0.14 mL/min, while the mean total salivary protein concentration was 1.02±0.36 mg/mL.

Table 1. Demographic and clinical characteristics of participants

Variable	Value
Number of participants	150
Age, mean ± SD	23.8 ± 3.1 years
Male, n (%)	78 (52.0)
Female, n (%)	72 (48.0)
Mean DMFT ± SD	3.2 ± 2.8
Salivary flow rate ± SD	0.31 ± 0.14 mL/min
Total protein ± SD	1.02 ± 0.36 mg/mL

Dental Caries Experience

Of the 150 participants, 31 (20.7%) were caries-free with a DMFT score of zero. Forty-six participants (30.7%) had low caries experience, with DMFT scores ranging from 1 to 3.
The remaining 73 participants (48.6%) had DMFT scores greater than 3 and were classified as having high caries experience.
The mean DMFT score among participants with DMFT >3 was 5.4±2.1.

Table 2. Distribution of participants according to DMFT

DMFT category	n	%	Mean DMFT ± SD
DMFT = 0	31	20.7	0.0
DMFT 1–3	46	30.7	1.9 ± 0.8
DMFT >3	73	48.6	5.4 ± 2.1
Total	**150**	**100.0**	**3.2 ± 2.8**

Comparison of Salivary Parameters by Caries Experience

Participants with high caries experience demonstrated a significantly lower mean unstimulated salivary flow rate than participants with low/no caries experience.
The mean flow rate was 0.27±0.13 mL/min in the high-caries group compared with 0.36±0.12 mL/min in the low/no-caries group.
This difference was statistically significant ($p<0.001$).
In contrast, the high-caries group demonstrated significantly higher total salivary protein concentration.
Mean protein concentration was 1.12±0.38 mg/mL in participants with high caries experience compared with 0.92±0.30 mg/mL among participants with low/no caries experience ($p=0.002$).

Table 3. Salivary parameters according to caries experience

Parameter	Low/no caries (n=77)	High caries (n=73)	p-value
Salivary flow rate (mL/min)	0.36 ± 0.12	0.27 ± 0.13	<0.001
Total protein (mg/mL)	0.92 ± 0.30	1.12 ± 0.38	0.002
DMFT	1.1 ± 0.9	5.4 ± 2.1	<0.001

Correlation between Salivary Flow and DMFT

Pearson correlation analysis demonstrated a significant inverse relationship between unstimulated salivary flow rate and DMFT.
The correlation coefficient was **r=-0.36 (p<0.001)**.
This finding indicates that participants with higher salivary flow rates tended to have lower dental caries experience.

Table 4. Correlation between salivary flow rate and DMFT

Variable	r	p-value
Salivary flow rate vs. DMFT	−0.36	<0.001

Correlation between Total Protein and DMFT

Total salivary protein concentration demonstrated a significant positive correlation with DMFT.
The correlation coefficient was **r=0.29 (p<0.001)**.
Thus, participants with greater dental caries experience tended to have higher total salivary protein concentrations.

Table 5. Correlation between total protein concentration and DMFT

Variable	r	p-value
Total protein vs. DMFT	+0.29	<0.001

Relationship between Salivary Flow and Protein Concentration
A weak inverse correlation was observed between salivary flow rate and total protein concentration.
The correlation coefficient was **r=-0.21 (p=0.010)**.
This suggests that lower salivary flow may be associated with greater protein concentration, potentially reflecting reduced dilution of salivary proteins.

Multiple Linear Regression
Multiple linear regression was performed to determine whether salivary flow rate and total protein concentration were independently associated with DMFT.
After adjustment for age, sex, sugar consumption and tooth-brushing frequency, salivary flow rate remained significantly negatively associated with DMFT.
Total protein concentration remained significantly positively associated with DMFT.
Frequent sugar consumption was also significantly associated with higher DMFT, whereas tooth brushing at least twice daily was associated with lower DMFT.
The overall model was statistically significant and explained approximately 23% of the variance in DMFT.

Table 6. Multiple linear regression analysis of factors associated with DMFT

Predictor	Standardized β	95% CI	p-value
Salivary flow rate	−0.29	−4.86 to −1.12	0.002
Total protein concentration	+0.22	0.61 to 2.84	0.003
Age	+0.11	−0.03 to 0.24	0.126
Male sex	+0.08	−0.31 to 0.94	0.321
Frequent sugar consumption	+0.24	0.38 to 1.72	0.002
Tooth brushing ≥2/day	−0.15	−1.46 to −0.12	0.021

Adjusted R^2=0.23; overall model p<0.001.

DISCUSSION
The present study investigated the relationship between unstimulated salivary flow rate, total salivary protein concentration and dental caries experience among young adults in Peshawar, Pakistan. The findings demonstrated two important associations: participants with greater caries experience had lower unstimulated salivary flow rates, while they had higher total salivary protein concentrations. Salivary flow rate was negatively correlated with DMFT, whereas total protein concentration was positively correlated with DMFT.
The inverse association between salivary flow rate and dental caries is biologically plausible. Saliva continuously contributes to the maintenance of oral homeostasis through mechanical clearance, buffering and remineralization. Reduced secretion can decrease the clearance of dietary carbohydrates and bacterial acids and may consequently prolong the period during which tooth surfaces are exposed to an acidic environment.
The present findings are consistent with evidence from Pakistan. Ansari et al. recently investigated unstimulated salivary parameters in 516 Pakistani schoolchildren and reported significantly higher salivary flow rates among caries-free participants compared with caries-active participants [9]. Although the participants in that study were younger and were recruited in Karachi, the consistency of the association strengthens the biological plausibility of the present findings.
The observed relationship between salivary flow and DMFT is also consistent with the established physiological role of saliva. Navazesh emphasized that saliva collection conditions can significantly affect measured flow rates, highlighting the importance of

standardized collection procedures in salivary research [5].

The second important finding was the positive association between total salivary protein concentration and dental caries experience. Participants with high caries experience had significantly higher mean protein concentration than participants with low/no caries experience. The interpretation of this finding requires caution. Total salivary protein is not a single biological molecule but represents a complex mixture of proteins with different and sometimes opposing functions. Some salivary proteins contribute to protection against caries, while others may reflect host responses or changes in the oral environment.

Previous systematic reviews have demonstrated inconsistent relationships between total salivary proteins and dental caries. Martins et al. found insufficient evidence to establish salivary proteins as definitive biomarkers, although some studies demonstrated associations between total protein concentration and caries [6].

More recent evidence has demonstrated that individual proteins may be more informative than total protein concentration. Ahmad et al. identified differences in several salivary proteins between caries-active and caries-free individuals. The proteins associated with caries included alpha-amylase, acidic proline-rich protein-1, histatin-5, lactoperoxidase and mucin-1, whereas carbonic anhydrase 6, proteinase-3 and statherin were more prominent among caries-free individuals [7].

The positive relationship between total protein and DMFT observed in this study could therefore represent a generalized change in salivary composition associated with the caries-active oral environment rather than a direct causal effect of proteins on caries.

An important consideration is the inverse relationship between salivary flow and protein concentration. In the present study, lower flow was weakly associated with higher protein concentration. This may be explained by dilution. Saliva consists predominantly of water, and a lower volume of water secreted per unit time can increase the measured concentration of proteins even without an increase in absolute protein secretion.

Therefore, the simultaneous assessment of flow rate and protein concentration represents a strength of the present investigation. If only protein concentration had been assessed, the observed increase might have been incorrectly interpreted as increased protein production.

The findings also support the concept that dental caries is associated with broader alterations in the salivary environment. A recent systematic review of salivary diagnosis of dental caries found that proteins were among the most commonly investigated salivary biomarkers and that multiple salivary molecules differed significantly between caries-active and caries-free individuals [8].

Similarly, recent evidence has demonstrated elevated total salivary protein content in caries-active populations. A systematic review focusing on children with mixed dentition reported that total protein content was higher among caries-active participants, although results for individual proteins such as alpha-amylase and carbonic anhydrase were inconsistent [10]. This supports the present finding while also emphasizing that total protein should be interpreted as a broad salivary characteristic rather than a disease-specific biomarker.

The regression analysis provided additional information. After adjustment for age, sex, sugar consumption and tooth-brushing frequency, salivary flow remained negatively associated with DMFT. This suggests that the relationship between salivary flow and caries was not completely explained by the behavioral variables included in the model.

Frequent sugar consumption was independently associated with higher DMFT, which is consistent with the established role of fermentable carbohydrate exposure in dental caries development [1,2]. This finding is important because salivary characteristics should not be interpreted independently from dietary behavior.

Tooth brushing at least twice daily was negatively associated with DMFT. Although brushing frequency is not a direct measure of brushing quality or fluoride exposure, this finding emphasizes the importance of preventive oral-hygiene practices.

The present study has particular relevance for Peshawar. Although several international studies have investigated salivary biomarkers, findings may vary between populations because of differences in diet, genetics, socioeconomic status, oral-hygiene practices, healthcare access and environmental conditions. Recent research has emphasized that genetic, dietary, oral-hygiene and demographic differences can contribute to variability in salivary biomarker findings [11].

The study therefore contributes preliminary evidence concerning the biological

characteristics associated with caries experience among young adults in Peshawar. It may also provide a foundation for future research investigating specific salivary proteins and oral microbial profiles in the same population.

The study has several strengths. First, both salivary quantity and composition were evaluated. Second, saliva collection was standardized with respect to time and pre-collection behavior. Third, dental caries was objectively assessed using the DMFT index. Fourth, potential behavioral confounders were considered in multivariable analysis. Finally, the study was conducted in a Pakistani population for which locally relevant evidence remains comparatively limited.

Nevertheless, several limitations should be acknowledged. The cross-sectional design prevents determination of causality. It cannot be established whether reduced salivary flow contributed to caries development or whether caries-associated behavioral and biological changes influenced salivary characteristics.

Second, total protein concentration is relatively nonspecific. Individual proteins may have different biological functions and may demonstrate different relationships with caries. Advanced proteomic approaches could provide substantially greater information than total protein measurement.

Third, DMFT measures cumulative caries experience rather than current caries activity. A filled tooth may represent a lesion that occurred several years earlier, while the index does not adequately capture early non-cavitated lesions. Future research could therefore incorporate ICDAS or another contemporary lesion-detection system.

Fourth, the participants were recruited from a dental institution in Peshawar. Consequently, they may not represent all young adults in the city. Community-based sampling would improve generalizability.

Finally, salivary flow and protein concentration can be influenced by hydration, stress, circadian rhythm, diet and medications. Although several factors were standardized or excluded, residual confounding cannot be completely eliminated.

Despite these limitations, the study demonstrates that salivary parameters are associated with dental caries experience and supports further investigation of saliva as a non-invasive source of information about oral health.

CONCLUSION

The present study demonstrated a significant relationship between salivary characteristics and dental caries experience among young adults in Peshawar, Pakistan.

Participants with greater dental caries experience had significantly lower unstimulated salivary flow rates and higher total salivary protein concentrations. Salivary flow rate was inversely correlated with DMFT, while total protein concentration demonstrated a positive correlation with DMFT.

The findings indicate that reduced salivary flow may represent an important host-related factor associated with increased caries experience. The increase in total salivary protein concentration may reflect changes in salivary composition or reduced dilution associated with lower flow.

Although these findings support the potential use of salivary parameters in caries-risk assessment, total protein concentration should not yet be regarded as a standalone diagnostic biomarker. Existing systematic reviews demonstrate substantial heterogeneity in the relationship between individual salivary proteins and dental caries [7,8].

Future studies in Peshawar should investigate specific salivary proteins, salivary microbiological profiles and longitudinal caries progression using larger community-based samples. Such research may help identify a combination of salivary biomarkers capable of predicting individuals at increased risk of dental caries.

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