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Research Article

Salivary Statherin as a Dental Caries Biomarker among a Sample of Adolescents in Baghdad City

Sarah Hikmat Yousif [1*], Alhan Ahmed Qasim [1]

[1] Department of Pediatric and Preventive Dentistry, College of Dentistry, University of Baghdad Baghdad, Iraq

* Corresponding author's email: sara.Hekmat1202a@codental.uobaghdad.edu.iq

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ABSTRACT

Background: Dental caries is a multifaceted disease that impacts teenagers worldwide. A combination of factors, including fermentable carbohydrates, acid-producing bacteria, saliva, and host-related characteristics, influences its development. Salivary Statherin controls microorganisms by aggregating them and preventing them from adhering to hard tissue and epithelium. Salivary Statherin may maintain tooth integrity.
Objective: This research investigated the potential of salivary Statherin as a biomarker for dental caries.
Subjects and Methods: This comparative (observational) research included 90 adolescents of both sexes aged 15 years old. Participants were divided into two groups: 30 individuals without caries, serving as the control group, and 60 individuals with caries, referred to as the study group. Following the World Health Organization guidelines, adolescents with varying caries experiences were further categorized. Thirty exhibited a moderate level of caries, with 1 to 3 affected teeth, while the remaining thirty were classified as having severe caries, with a Decay-Missing-Filled Teeth (DMFT) score greater than 10. The DMFT Index measured caries experience—salivary Statherin analysis from unstimulated saliva. Height and weight were measured, and body mass index was calculated. classifying adolescents as normal weight, overweight, or obese.
Results: Statherin levels decreased with the severity of caries. There was a significant difference in the severe caries group, Statherin levels varies with different BMI group (P value ≤ 0.05).
Conclusions: Caries severity reduces salivary Statherin. In severe caries patients, Dietary status and dental caries severity affected Statherin levels. No association was found between Statherin levels and salivary pH or flow rate across different BMI group or caries severity groups. However, salivary Statherin may play a role in maintaining tooth integrity.

Introduction

Caries is a biofilm-mediated, sugar-driven, complex, dynamic illness distinguished by phasic demineralization and remineralization of tooth hard tissues (1,2).
Several variables can influence the progression of tooth decay. Caries develop when pathogenic bacteria and a substrate coexist on a vulnerable tooth surface. Bacteria transform the substrate into acid in this environment, leading to tooth decalcification (3-5).

Adolescence is a multi-system shift from childhood immaturity and social dependence to adulthood, with the objective and expectation of reaching one's developmental potential, establishing personal agency, and adopting societal responsibility (6). One-third of a person's total growth occurs throughout adolescence, which begins with the onset of puberty. At this age, cultural and individual variations are pronounced (7). Everyone is at risk for acquiring dental caries, but adolescents are at a higher risk. There is a correlation between the sugar intake of adolescents and dental caries (8). Studies have shown that teens had a higher prevalence of dental caries than other age groups (9,10). However, the causes linked with dental caries, particularly among adolescents, are poorly understood. This investigation sought to investigate parameters linked with dental caries in connection to teenagers' nutritional status.

Diet and nutrition can influence the formation and integrity of the oral cavity, as well as the advancement of oral disorders (11-13). The relationship between body weight and dental caries remains a subject of debate (14). While a connection between obesity and dental decay has been established in adults, the association in adolescent populations is less clear. This study explored the link between these two significant factors in adolescent samples, with the aim of supporting the planning and promotion of adolescent healthcare.

Saliva is a viscous, aqueous, electrolyte- and protein-rich fluid that plays a role in regulating oral microbiota, protecting tooth enamel, and other oral tissue defenses. Salivary proteins serve as precursors for acquired enamel pellicles (AEP), playing a vital role in protecting oral surfaces. Given its noninvasive nature and ease of collection, saliva has garnered attention from researchers as a potential diagnostic tool. Additionally, saliva contains specific biomarkers that are related to health or disease (16). Plaque is eliminated by salivary proteins, which also delay demineralization, promote remineralization, neutralize acids, and prevent infection (17,18). Lysozyme, lactoperoxidase, immunoglobulins, lactoferrins, mucins, albumin, histatins, defensins, Statherin, and cathelicidin are saliva proteins that protect oral tissue (19).

Early pellicle proteins, proline-rich proteins, and Statherin may produce a protective layer for the oral cavity. By attracting calcium ions and delaying demineralization, pellicle proteins aid salivary calcium and phosphate ions in remineralizing enamel. Salivary (glyco) proteins inhibit the attachment and growth of oral bacteria to the enamel pellicle (20,21).

Rich in tyrosine and dephosphorylated, Statherin is an asymmetric salivary peptide with 43 residues that is released by the acinar cells of the parotid and submandibular salivary glands. The usual range of Statherin concentrations in human saliva is 10–40 μM. (22). Hydroxyapatite (HA) adsorption is the primary function mediated by the prime amino acid sequences, which helps with in situ enamel biomimetic remineralization (22). Therefore, Statherin is important for controlling enamel mineralization, and further study is necessary to determine whether it may be regarded as a powerful salivary indicator of dental cavities (22). Salivary Statherin remineralizes and captures calcium ions to protect tooth surfaces (23). The aggregation of microorganisms restricts bacterial and fungal colonization by limiting adhesion to hard tissue and epithelium (24).

No research has linked Statherin to teenage tooth decay. Therefore, Statherin cannot predict the risk of caries in adolescents without more studies. Along with offering personalized dental care, salivary proteins like Statherin hold potential for identifying associated risk factors, estimating the possibility of dental cavities, and facilitating dental screenings. This study is designed to explore the potential of Statherin as a biomarker for dental caries, particularly in relation to the BMI status of adolescents.

Subjects and Methods

This observational research compared both genders of adolescents aged 15 years recruited from the Teaching Hospital / University of Baghdad, College of Dentistry, and various governmental and private intermediate schools in varying Baghdad areas.

Biochemical analyses were carried out within the study's designated time frame. The University of Baghdad's College of Dentistry's ethical approval committee authorized it. (The ethics committee gave its approval to this study, Ref. number: 482, Date: 19-1-2022). Prior to data collection, legal permission was obtained from Ministry of Education. In order to get authorization to participate in this research, a distinct consent form was also created and sent to every participant. This research was conducted over the period from 12 Dec 2021 to 30 Apr 2022.

The software "G power 3.1.9.7" was created by Franz Faul, a professor at the University of Kiel in Germany, to determine the required sample size. With a big effect size of 0.40 across six groups and an alpha error of 0.05 for two-sided testing, the research showed that a sample of 82 individuals was required to reach a study power of 90%. The overall sample size increased to 90 people when a 10% margin of error was included. There were three categories for the effective size (F): small (0.1), medium (0.25), and big (0.4).

Following a thorough examination of 380 adolescents aged 15 from both genders, 90 individuals were selected and categorized into two groups based on the 2013 WHO regulations. Among them, 60 participants had a history of caries, forming the study group, while 30 had no caries experience, serving as the control. The adolescents with differing experiences of caries were further divided, with thirty having moderate carious teeth (Decay-Missing-Filled Teeth score of 1-3) and thirty exhibiting severe carious teeth (Decay-Missing-Filled Teeth score exceeding 10).

The participants were divided into three groups based on their BMI: those with Normal BMI, overweight, and Obese.

Qualification criteria:

- Adolescents who do not have systemic impacts on salivary secretion or local illnesses.
- Patients expressed their willingness to give consent for participation in the study.

Exclusion criteria:

- Adolescents suffering from systemic illnesses.
- Individuals on a restricted diet.
- Patients who received fluoride as their teeth were growing.
- An adolescent who has structural dental abnormalities and has started orthodontic treatment.

The dental health assessment involved diagnosing and reporting dental caries experience using the DMFT index, following the criteria established by WHO in 2013. A WHO probe, specifically the CPI "Community Periodontal Index" probe, along with a flat mouth mirror, was utilized for examining patients' teeth.

The assessment of Body Mass Index (BMI) for adolescents involves calculating a value based on weight and height, utilizing the formula:

$$BMI = \frac{weight\ (kg)}{height^2\ (m^2)}$$

This BMI measurement is represented in various charts that are specific to age and gender, known as CDC growth charts.

Sample collection will involve gathering morning saliva samples from each adolescent between 9 and 12 A.M. Unstimulated saliva will be collected under standardized conditions, adhering to the established guidelines (27). Salivary Statherin levels were determined using enzyme-linked immunosorbent assay (ELISA) kits for analysis and detection.

Salivary flow rate

The flow rate of salivary was calculated by dividing the collected saliva volume, measured in millilitres (ml), by the duration of the collection period, expressed in minutes. (28).

$$Salivary\ flow\ rate\ \left(\frac{mL}{min}\right) = \frac{Volume\ (mL)}{Time\ (minute)}$$

Salivary pH

A digital pH meter was used to measure the pH of resting saliva. The pH meter was calibrated in accordance with the manufacturer's guidelines.

Statistical analysis

One-way analysis of Variance (ANOVA) and the Games-Howell post hoc test were employed to describe, analyze, and present the data, utilizing the Statistical Package for Social Science (SPSS version 22, Chicago, Illinois, USA) for cases involving unequal variance.

Results

Table (1) shows that salivary Statherin is decreased with increasing caries severity. There is a significant result in normal BMI, obese groups, and the total sample among caries severity group while in overweight is not significant.

The level of salivary Sthatherin among BMI groups rises, and there is a significant difference only in the total sample and in the severe caries group among nutritional status. In contrast, in other caries groups, it is not significant.

Table (2) presents multiple pairwise comparisons of salivary statherin levels concerning caries and nutritional status, utilizing the GamesHowell post hoc test. The findings reveal a significant difference in salivary Statherin levels between individuals of normal weight and those who are obese. Additionally, a significant difference is noted between the groups with no caries and those with severe caries, while the remaining comparisons do not show significant results.

The results indicate that salivary flow rate (SFR) and salivary pH exhibit a weak negative correlation with salivary Statherin, without any significant relationships, except in the severe caries group, where a strong negative correlation is observed between salivary Statherin and SFR. In both the free and severe caries groups, salivary Statherin demonstrates a weak negative correlation with salivary pH, as illustrated in Table (3).

Additionally, findings indicate a weak negative and non-significant correlation between salivary Statherin, pH, and flow rate across various BMI groups. However, a weak but significant negative correlation with salivary pH was observed within the normal weight group. Similarly, in the obese group, salivary Statherin showed a weak significant negative correlation with salivary flow rate, as presented in Table (4).

Table 1: Descriptive analysis and statistical testing of Statherin levels across BMI status and caries severity.

Caries severity groups	BMI	Mean	±Standard Error	F	Pvalue
Free of caries group				1.324	0.283
	Normal BMI	27.054	1.612		
	Overweight	28.157	2.655		
	Obese	30.741	1.512		
Mild caries (1-3) group				1.211	0.314
	Normal BMI	25.114	1.388		
	Overweight	26.151	3.183		
	Obese	30.280	1.713		
Severe caries (Decay < 10) group				5.246*	0.010*
	Normal BMI	20.853	0.669		
	Overweight	22.867	0.567		
	Obese	25.251	0.302		
Total among BMI groups				8.224*	0.001*
	Normal BMI	23.749	0.741		
	Overweight	24.976	1.201		
	Obese	29.528	1.030		
Total among caries severity groups				15.327*	0.000002*
	Free of caries	28.5901	1.045		
	Mild caries	26.2517	1.200		
	Severe caries	21.8970	0.489		

BMI	Caries	F	Pvalue
Normal BMI	Free-mild-severe	8.063*	0.001*
Overweight	Free-mild-severe	1.685	0.208
Obese	Free-mild-severe	4.079	0.045*

* A p-value of less than or equal to 0.05 indicates a significant difference

Table 2: Statherin's Pairwise Comparisons by Caries and Nutritional Status (Games-Howell).

Caries severity category	Group	Mean difference	P value
Severe caries (Decay < 10) group	Normal weight X Overweight	-2.014	0.139
	Normal BMI X Obese	-4.398	0.018*
	Overweight X Obese	-2.383	0.379
Total among caries severity groups	Normal BMI X Overweight	-1.227	0.662
	Normal BMI X Obese	-5.779	0.000141*
	Overweight X Obese	-4.552	0.05102
BMI Categories	Groups	Mean difference	Pvalue
Total among BMI groups	Free X Mild	2.338	0.313
	Free X severe	6.693	0.00000*
	Mild X severe	4.355	0.052023
Normal BMI	Free X Mild	1.940	0.638
	Free X severe	6.201	0.006*
	Mild X severe	4.261	0.05121
Obese	Free X Mild	0.462	0.978
	Free X severe	5.491	0.012*
	MildXsevere	5.029	0.087

*P value <= 0.05 significant

Table 3: Correlation of Salivary pH, Flow Rate, and Statherin with Caries Severity Groups.

Groups of caries severity		Salivary pH		Salivary flow rate	
		relation	Pvalue	relation	P value
Free of caries group	Statherin	-0.363	0.049*	-0.215	0.183
Mild caries (1-3) group	Statherin	-0.235	0.211	-0.197	0.297
Severe caries (Decay < 10) group	Statherin	-0.400	0.011*	-0.390	0.033*

*P value <= 0.05 significant difference

Discussion

According to the current study, salivary Statherin levels decreased with increasing caries severity. There is a significant result in normal weight, obese groups, and the total sample among caries severity groups. Because Statherin helps build the acquired enamel pellicle, which may affect tooth bacteria colonization. Statherin's basic amino acids inhibit sugar metabolism by bacteria, affecting dental plaque (29). Statherin has beneficial antimicrobial effects. It appears to limit microbial colonization of the mouth and modulate salivary calcium phosphate chemistry. This effect, in turn, maintains salivary supersaturation and tooth mineralization. Dental caries is prevented by these actions (30). This finding was consistent with the findings of

Table 4: Correlation between salivary PH, salivary flow rate, and salivary Statherin in different BMI groups

BMI groups		Salivary PH		Salivary flow rate	
		relation	P value	relation	P value
Normal BMI	Statherin	-0.340	**0.011***	-0.236	0.083
Overweight	Statherin	-0.304	0.140	-0.041	0.845
Obese	Statherin	-0.070	0.770	-0.421	**0.036***

*P value <= 0.05 significant difference

Angarita-Daz et al. (31), who discovered that Statherin expression was significantly higher in saliva samples taken from healthy children than from children with moderate or severe caries (ICDAS > 3). Furthermore, a study conducted among preschool children in Iraq discovered that protein levels were higher in caries-free children than in caries-active children (32). Vitorino et al. (33) found a strong link between a child's high level of Statherin and their lack of tooth decay. This finding contrasts with the study of Preethi et al. (34), who discovered that children with caries had significantly higher salivary protein levels than children without caries. Ahmadi-Motamayel et al. (35) conducted a study among healthy 15- to 17-year-old students. They discovered higher levels of total protein but statistically insignificant differences between the caries-active and caries-free groups.

There is a correlation between the BMI and salivary sthatherin levels; however, no such correlation exists between the entire sample and the severe caries group; moreover, no such correlation exists between the other caries groups and nutritional status. This could be attributed to the salivary Statherin's defense against the free diffusion of highly concentrated acids on tooth surfaces (36) due to frequent snacking, sugary drinks, and foods, and snacking between meals can cause dental caries and obesity (37). These findings are consistent with an Iraqi study, which found that obese children had higher amounts of total salivary protein than their normal-weighted counterparts (38). However, this finding was supported by another study by Acevedo, which found that overweight and obese children, compared with the control group, had higher protein content (39). According to the literature, there are no previous studies concerning especially salivary Statherin and BMI.

The data reveals a significant weak negative correlation between salivary Statherin and pH across the caries severity groups. Additionally, in the severe caries group, a strong significant negative correlation exists between salivary Statherin and salivary flow rate (SFR). A decrease in salivary flow rate and pH is observed as both nutritional status and caries severity worsen. Consistent with these findings, research by Bakkal and Kargul identified a weak correlation between unstimulated whole saliva pH and caries activity (40). In both groups, an increase in salivary flow rate was observed, while protein concentration showed a decrease. It seems that the concentration of proteins is inversely related to the salivary flow rate, which makes sense, given that proteins in saliva are diluted.

Conversely, a lower pH of salivary was associated with increased total protein concentrations. These results align with the findings of Vitorino et al., who reported higher total concentrations of protein in the caries-active groups (41). It is possible that lower anti-proteolytic mechanisms or enhanced proteolytic activity are connected with the greater protein levels seen in the caries-active group. Therefore, it might be interpreted as a protective response when children with caries have saliva with a lower pH and a higher protein content. Additionally, there is an inverse relationship between protein content and salivary flow rate. These observations align with Bhalla et al.'s assertion that an increase in salivary flow rates leads to the dilution of salivary proteins (42).

The study's limitations include the fact that dental caries were evaluated using only visual and tactile responses and no radiographic data. Consequently, the prevalence of dental caries was likely underestimated. The study found it difficult to establish a connection between frequent sugar consumption and dental caries. This may have been due to the study's cross-sectional design; the association could have been demonstrated through a case-control or cohort study.

Conclusion

Adolescents are affected by dental caries. The current research concluded that salivary Statherin declines with caries severity due to its protective function in contradiction to the free diffusion of highly concentrated acids on the tooth surface. It is hypothesized that salivary Statherin may contribute to protecting the teeth from decay caused by acidic bacteria secretions on the surface of the enamel. A weak negative correlation, which is not statistically significant, is present between salivary Statherin and both pH of salivary and salivary flow rate within the different caries severity groups.

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Conflict of Interest

Authors declare no conflict of interest.

Data availability

Data are available upon reasonable request

ORCID

Sarah Yousif 0000-0002-4507-1406
Alhan Qasim 0000-0003-0682-664X

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