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Research Article

Role of Ramadan Fasting on Secretory IgA and Statherin Levels in Individuals with Dental Caries

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Abstract

Background:
Tooth decay happens when bacteria in the mouth create acid from carbohydrates that can be fermented. Along with factors related to the person (like genetics or oral hygiene habits) and saliva, this process leads to cavities. During Ramadan fasting, when eating habits change, there could be effects on health, particularly on the salivary biomarkers that are important for dental well-being.

Objectives: This cross-sectional study aimed to detect the activity and concentrations of salivary levels of secretory immunoglobulin A and Statherin during and after Ramadan fasting and to evaluate the association between these biomarkers with dental caries.

Methods: The study comprised 40 individuals, aged 20-25 years, diagnosed with dental caries. Participants were assessed for periodontal parameters using the Plaque Index and the Gingival Index. Saliva samples were collected during the fourth week of Ramadan fasting and two weeks after Ramadan fasting. The concentrations of both secretory IgA and Statherin were measured in salivary samples using Enzyme-Linked Immunosorbent Assay.

Results: A significant decrease in the mean concentration of secretory immunoglobulin A was observed during Ramadan fasting (2.14±0.21 ng/L) compared to post-fasting (3.34±0.35 ng/L) (p=0.001). However, there was a non-significant difference (p=0.05) in slathering levels between the fasting state (2.25±0.18 ng/L) and the post-fasting state (2.85±0.22 ng/L). No statistically significant difference was found concerning both the Plaque Index and the Gingival Index within fasting and post-fasting states.

Conclusion: Low concentration of sIgA and Statherin during Ramadan fasting may indicate altered salivary gland activity or systemic response to fasting, potentially affecting oral health.

Keywords: Dental Caries; Fasting; Gingival Index; Plaque Index; Secretory immunoglobulin A (sIgA); Statherin,

Introduction

Ramadan, the ninth month of the lunar calendar, lasts 29–30 days. During this period, Muslims fast from dawn until sunset, refraining from food and drink for 11–18 hours daily. This change in eating habits and alterations in relaxation and exercise routines disrupt daily rhythms (1). Religious fasting has implications for physiology and disease pathogenesis mechanisms (2). Maintaining good oral hygiene can significantly mitigate dental issues, habits such as neglecting nighttime brushing, overeating, or consuming high-fat meals can lead to oral disorders. These include dental caries, gingivitis, and periodontitis (3). Dental caries is a prevalent and complex global oral health concern, resulting from interactions among acid-producing bacteria, fermented carbohydrates, host factors, and saliva (4,5).

Saliva plays a pivotal role in oral health and physiology. It's also a trusted tool for detecting biomarkers indicative of host protective mechanisms. Salivary proteins help clean teeth, prevent abrasion, delay demineralization, facilitate remineralization, neutralize acids, and protect the oral cavity from infections (6).

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Components like Lysozyme, lactoferrins, lactoperoxidase, immunoglobulin, albumin, mucins, histatins, statherin, defensins, cathelicidin LL-37, and other immunoglobulins shield oral tissues (7,8). Secretory IgA (sIgA) is an antibody prevalent in mucous membranes, including those in the oral cavity, respiratory, and digestive tracts. As the primary immunoglobulin in saliva, sIgA is vital for defending the oral cavity against microbial invasions. Bacteria, especially Streptococcus mutans, form biofilms (plaque) on teeth and are the main cause of fermenting dietary sugars. The resultant acids, especially lactic acid, demineralize tooth enamel, leading to cavity formation (8,9). sIgA antibodies in saliva combat dental caries in several ways: by binding to bacterial toxins, enzymes, and adhesins, inhibiting bacterial adherence and colonization, causing bacterial agglutination, and tagging bacteria for immune system destruction (9). Additionally, sIgA forms a barrier on the tooth, impeding bacterial penetration (10). Statherin, a 43-amino acid peptide weighing 5.4 kDa, protects teeth by capturing calcium ions and fostering remineralization. It also manages bacteria by clustering them, preventing them from sticking to hard tissues and epithelium (11). Statherin is increasingly recognized as a potent salivary

indicator of bacterial infection and dental caries (12). Fasting has the potential to alter immunological functions. It augments catabolism and macrophage activity through cellular breakdown (13). While fasting boosts B cell-mediated immunity, its effects on cell-mediated immunity are less clear. It enhances the activity of neutrophils (bactericidal), monocytes, and natural killer cells (14). This study aims to detect the activity and concentrations of secretory IgA and statherin levels in saliva during and after Ramadan fasting and evaluate the association between these biomarkers and dental caries.

Materials and Methods

Subjects

This cross-sectional study included forty participants (20 females and 20 males) who attended the Dental College Teaching Hospital/ Baghdad University and Specialized Dental Center /Al-Sadr City for treatment and follow-up of tooth decay. Their ages ranged from 20 to 25 years. During the patient's follow-up, a second saliva sample was retaken two weeks post-fasting.

Ethical approval

The study protocol was approved by the scientific committee at the Basic Sciences Department/College of Dentistry/University of Baghdad, on 1/4/2023 (Project No. 824823). All patients were given detailed information about the study's objectives and informed consent was signed to represent the patient's acceptance of being involved in the study.

Inclusion criteria:

1. Individuals suffering from tooth decay between the ages of 20-25 years.
2. Individuals who take their last meal two hours before sunrise (at dawn).
3. Those who do not mind following up after fasting.

A follow-up was conducted for this category two weeks after Ramadan fasting.

Exclusion criteria

1. Individuals with systemic, chronic, autoimmune diseases or hypersensitivity reactions, in addition to those having DM, periodontal diseases, or any inflammatory conditions.
2. Non-fasting subjects.
3. Individuals with structural dental defects.
4. Patients undergoing caries treatment.
5. Smoking subjects.

Oral examination:

During the study, each subject underwent a clinical examination conducted by a dentist. This included assessing the periodontal status of all teeth using a periodontal probe. The key periodontal parameters measured were the plaque Index (PI) and the gingival Index (GI).

The presence of plaque is a primary factor in the development of cavities (dental caries) and gingival disease (periodontal disease). When using the Plaque Index, a dental professional typically examines the teeth and assigns a score based on the thickness of plaque and the area it covers. The scoring ranges from 0 (no plaque) to a higher number indicating a thicker and more extensive plaque covering. The Gingival Index system was used to evaluate gingival inflammation, with the presence of inflammation on two surfaces of each tooth being noted. The scoring system for this index ranged from 0, indicating no plaque, to 1, signifying the presence of gingival inflammation (15).

Saliva Collection

One to three milliliters of whole unstimulated saliva were collected for the same participant at two different times; the first, during the fourth week of fasting and the second, two weeks after Ramadan. The samples were gathered between 9 a.m. and 12 p.m. After collection, each sample was placed in a sterile plain tube and centrifuged at 2000 rpm for 15 minutes. The supernatant was then transferred to an Eppendorf tube. All samples were subsequently stored in a deep freezer at -80°C, until analysis (9). Enzyme-linked immunosorbent Assay (ELISA) was employed for the detection of pro-inflammatory biomarkers using the Human ELISA quantitative immunoassay kit (Secretory IgA/Lot No: E23DYH836, Feiyuo company, China) and (Statherin/Lot No: E23DAJ660, Feiyuo company, China) in saliva samples. The readings were obtained using an ELISA reader from BioTek (USA). **Principle of the Procedure:** This kit uses sandwich enzyme immunoassay. This package includes a microtiter plate pre-coated with either Secretory IgA and Statherin antibody. Microtiter plate wells containing standards or samples get a biotin-conjugated Secretory IgA and Statherin antibody. Incubate microplate wells with avidin-HRP. TMB substrate solution only colors wells containing Secretory IgA and Statherin, biotin-conjugated antibodies, and enzyme-conjugated avidin. Sulfuric acid blocks the enzyme-substrate reaction, and the color change is measured at 450 nm±10 nm. The concentration of biomarkers in the samples is then determined by comparing the optical density (OD) of the samples to the standard curve (16).

Statistical analysis

The data processing utilized Statistical Package for Social Sciences (SPSS) version 26 and Microsoft Excel 2010. Statistical analyses included Chi-squared and *t*-test, as well as Wilcoxon Signed Ranks tests. The significance level was set at $P<0.05$ for significant results.

Results

The study includes 40 young individuals between the ages of (20-25) years. The data revealed that there was a non-significant difference between female and male participants regarding their mean age as it was found to be (22.80±1.936) years for the females and (22.85±2.007) years for the males. Statistical analysis

(*P*>0.05) indicated that the observed differences in mean age for both sex was not statistically significant. According to the findings of this study, there is no significant difference (p > 0.05) in both the Plaque Index (PI) and the Gingival Index (GI) between the fasting and post-fasting follow-up periods. The mean values for the PI were (0.18±0.15) for the fasting group and (0.16±0.15) for the post-fasting group. Similarly, the mean values of the GI for both periods were (0.21±0.23) and (0.24±0.11), respectively, as shown in Table 1.

Table 1: Levels of Plaque Index (PI) and Gingival Index (GI) parameters in study groups (fasting, post-fasting)

Variable	Study group		*t*- test	*P*-value
	Fasting	Post-fasting		
	mean±SD	mean±SD		
Plaque index (PI)	0.18±0.15	0.16±0.15	0.455	0.651
Gingival index (GI)	0.21±0.23	0.24±0.11	0.623	0.537

The results for sIgA are presented in Table 2. The level of sIgA (ng/L) decreased in the fasting group (2.14±0.21 ng/L) compared to the subsequent post-fasting measurement (3.34±0.35 ng/L), with a statistically significant difference (p=0.001),

Table 2: The changes in salivary concentration of sIgA (ng/L) in individuals with dental caries (fasting and post-fasting).

Wilcoxon Signed Ranks Test	sIgA concentration (ng/L)	
	Fasting	Post-fasting
mean±SD	2.14±0.21	3.34±0.35
p-value	0.001	

The results in Table 3 indicated a non-significant decrease in statherin levels between the fasting group compared to post-fasting one. The mean±SD values were (2.25±0.18) and (2.85±0.22), respectively, (*P*>0.05).

Table 3: The changes in Statherin (ng/L) levels among individuals with dental caries(fasting and post-fasting).

Wilcoxon Signed Ranks Test	Statherin level (ng/L)	
	Fasting	Post-fasting
mean±SD	2.25±0.18	2.85±0.22
p-value	0.05	

Discussion

The present study consisted of 40 individuals diagnosed with dental caries, equally divided into males and females. This intentional gender parity in the study design was crucial to offset potential biases associated with gender variations. As posited by McGregor *et al*. (2016) upholding a gender-balanced sample is essential due to the potential influence of gender discrepancies on clinical outcomes and biomarker calculations (17).

The main focus of this study revolved around participants aged between 20 and 25 years old, which's a demographic for understanding the development and progression of dental and oral conditions. This age group is particularly important because it signifies a period of transition. As people move from adolescence to adulthood, there are often changes in their preferences, dental care habits, and overall health behaviours that can be observed (18). The mean ages for both genders were very similar; females had an age of 22.80±1.936 years, while males had an age of 22.85±2.007 years. This similarity in ages ensures that any potential variations related to age are equally represented in both genders, thus enhancing the reliability of the findings regarding biomarkers.

Nevertheless, it's pivotal to acknowledge that when applying these findings to populations outside this age bracket, there may be variations in outcomes. Analyzing differences between this young cohort and middle-aged or senior groups could offer deeper insights into the development and presentation of oral ailments (19).

The overall well-being of a person's health is closely connected to the buildup of dental plaque. Within the field of study, the Plaque Index (PI) and Gingival Index (GI) play roles as key measures in evaluating oral hygiene and gingiva health (15).

As elucidated by Jasim *et al*. (2023) dental plaque, predominantly a bacterial biofilm, is identified as the chief instigator of dental caries and periodontal diseases (16). Consequently, fluctuations in the Plaque Index (PI) can shed a light on susceptibility to certain oral maladies. The recorded PI values in this study, for both fasting and post-fasting periods, appear relatively subdued (20).

A heightened GI often signals an escalation in inflammation, potentially pointing to susceptibilities towards gingival and periodontal complications. The minimal discrepancies in GI values between the two fasting phases, while not statistically profound, hint at sustained gingival health throughout (21).

Previous research has documented shifts in PI and GI values explicitly during Ramadan. Peedikayil and Narasimhan, (2019) noted a modest, yet statistically significant surge in periodontal inflammation during fasting, possibly tied to alterations in dietary habits or diminished oral hygiene practices (22). Contrarily, Telgi *et al*. (2013) discerned no remarkable variances in GI values throughout fasting, an observation aligning with the current study's outcomes (23). These disparate findings across studies may stem from diverse factors, including research methodologies, cohort sizes, and divergent dental care habits across populations.

While this study did not delve deeply into the underlying mechanisms, it's plausible that fasting might influence oral hygiene practices, the composition of saliva, and bacterial dynamics, thereby potentially affecting both PI and GI (24). The modest variations observed in this study's outcomes suggest that any potential impacts of fasting were either marginal or effectively counteracted by the prevailing oral conditions.

Secretory immunoglobulin A (sIgA) plays a role in protecting the mucosa from infections. The concentration of sIgA can potentially impact a

person's vulnerability to health issues (9). It plays several primary roles, including neutralizing invasive pathogens, preventing their adhesion to epithelial cells, and aiding in the clearance of antigens and microbes from the mucosal surface. The current study observed a significant drop in Statherin levels during fasting compared to the after fasting groups.

Decreased levels of sIgA could potentially compromise the body's defence mechanisms, making the oral mucosa more susceptible to pathogens (25). When it comes to sIgA production and secretion, both fasting and diet can have an impact. Fasting has been observed to reduce sIgA levels as it puts strain on the system. However, once food is consumed after a period of fasting, there is a boost in sIgA synthesis. This could be attributed to increased metabolic activity and an immune response to antigens and microbes that are introduced through feeding (26).

The findings of this study were in agreement with Nagai *et al*. (2019) who noted diminished sIgA levels during food restriction phases (27). They inferred that metabolic shifts and decreased antigenic stimulation of the oral mucosa's immune system contributed to this decline. However, Pietrzak (2020), postulated that extended fasting consistently elevated sIgA levels, highlighting potential immunoregulatory benefits of fasting (28). The discrepancies observed across these studies might stem from various factors, including the duration of fasting, post-fasting dietary attributes, and individual immune response variations. Identifying variations in sIgA levels due to fasting could offer valuable insights into periods marked by heightened vulnerability to oral infections. Therefore, devising strategies to bolster oral immunity, especially during periods of increased vulnerability like extended fasting, might be beneficial.

Statherin plays a crucial role in maintaining oral health. When present in saliva in large amounts, it performs functions for dental well-being. Its primary responsibility is to regulate the levels of calcium and phosphate ions in saliva to prevent them from forming calcium phosphate salts (29). Statherin helps maintain the balance between tooth demineralization and remineralization, which are responsible for the development and prevention of cavities. Moreover, statherin possesses properties that inhibit the growth of bacteria while also aiding their attachment to tooth enamel. Understanding the composition and growth patterns of biofilm is imperative for maintaining oral health (30).

This study noted a considerable non-significant decrease in Statherin levels during fasting compared to after fasting. The diminished levels might imply a reduced capability of saliva to sustain calcium and phosphate ion supersaturation during fasting. This could heighten tooth enamel's susceptibility to demineralization, especially if fasting extends without proper hydration and nutrient replenishment. Additionally, a decrease in Statherin might alter microbial colonization patterns on tooth surfaces, potentially influencing plaque's composition and properties (31).

Similarly, A study by Chennaoui et al. (2009) who, when examining Ramadan fasting effects on salivary components, identified similar Statherin level reductions, suggesting possible links to diminished salivary flow and glandular secretion changes during fasting (32). In contrast, Besbes et al. documented non-significant shifts in Statherin levels in individuals practicing intermittent fasting. Such differences could stem from variations in, during and after fasting, dietary habits (33).

Conclusion

The study observed salivary levels of sIgA elevated during the fasting period, whereas statherin levels were higher post-fasting. These biomarkers play crucial roles in maintaining oral health by defending invasions and regulating the balance of calcium and phosphate ions in saliva.

Limitation:

Challenges encountered in this study primarily stem from issues related to participant compliance and sample collection. Locating suitable participants was complicated by the fact that some did not adhere to oral hygiene instructions, crucial for ensuring valid sample collection. Additionally, inconsistent adherence to fasting among participants posed further difficulties, leading to considerable effort in collecting and validating samples.

Authors' declaration:

We confirm that all the Figures and Tables in the manuscript are mine/ ours. Besides, the authors have signed an ethical consideration's approval-Ethical Clearance. The project was approved by the local ethical committee in the College of Dentistry/University of Baghdad, according to the guidelines on biomedical research, The license has the code number 824823 dated 1/4/2023

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دور صيام رمضان في مستويات الغلوبولين المناعي A الإفرازي (sIgA) والستاثيرين لدى الأفراد المصابين بتسوس الأسنان

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الخلاصة

خلفية البحث: يحدث تسوس الأسنان عندما تنتج البكتيريا الموجودة في الفم أحماضا من الكربوهيدرات القابلة للتخمير. هذا، بالإضافة إلى العوامل الفردية مثل الجينات أو عادات النظافة الفموية، فضلا عن اللعاب، يؤدي إلى تكوين التجاويف. خلال صيام شهر رمضان، يمكن أن تؤثر التغييرات في عادات الأكل على الصحة، وخاصة المؤشرات الحيوية اللعابية المهمة لصحة الأسنان.

الاهداف: هدفت هذه الدراسة المقطعية إلى الكشف عن نشاط وتركيزات مستويات اللعاب من الغلوبولين المناعي الافرازي A (IgA) والستاثيرين خلال فترة الصيام في رمضان وبعدها، وتقييم العلاقة بين هذه المؤشرات الحيوية وتسوس الأسنان .

طرق العمل :تضمنت الدراسة 40 فردا، تتراوح أعمارهم بين 20 و25 عاما، تم تشخيصهم بتسوس الأسنان. تم تقييم المشاركين لمعلمات اللثة باستخدام مؤشر البلاك (PLI) ومؤشر اللثة. تم جمع عينات اللعاب خلال الأسبوع الرابع من شهر رمضان ومن ثم خلال أسبوعين بعد الصيام. تم قياس تركيزات الغلوبولين المناعي الافرازي A (IgA) والستاثيرين في عينات اللعاب باستخدام تقنية الفحص المناعي الإنزيمي المرتبط(ELISA)

النتائج: لوحظ انخفاض كبير في متوسط تركيز sIgA خلال صيام رمضان (2.14 ± 0.21) مقارنة بعد الصيام (3.34 ± 0.35) (p = 0.001). ومع ذلك، كان هناك فرق غير كبير (ع = 0.05) في مستويات ستاثرين بين مجموعة الصيام (2.25 ± 0.18) ومجموعة ما بعد الصيام (2.85 ± 0.22). لم يتم العثور على فروق ذات دلالة إحصائية فيما يتعلق بكل من مؤشر البلاك (PI) ومؤشر اللثة (GI) داخل مجموعات الدراسة.

الاستنتاجات: قد يشير انخفاض تركيز sIgA و Statherin خلال صيام رمضان إلى تغير نشاط الغدد اللعابية أو استجابة جهازية للصيام، مما قد يؤثر على صحة الفم.

مفتاح الكلمات: صيام رمضان، الغلوبولين المناعي A الإفرازي ، ستاثرين، مؤشر البلاك (PI)، مؤشر اللثة (GI)، تسوس الأسنان..