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Research Article

The Impact of Ramadan Fasting on Oral Health Biomarkers Linked to Dental Caries

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ABSTRACT

Background: Dental caries is a prevalent oral health problem, with saliva playing a pivotal role in maintaining oral hygiene. Fasting during Ramadan offers a unique scenario where changes in dietary habits, salivary flow, and pH levels might affect dental health markers.

Aim of the Study: This study aims to understand the implications of Ramadan fasting on various dental biomarkers, such as pH, Salivary Flow Rate (SFR), Glucosyltransferase (GTF), and Interleukin-8 (IL-8) levels in individuals with dental caries.

Subjects and Methods: A longitudinal observational study was conducted on 40 participants with dental caries, evenly distributed between males and females, aged between 20-25 years, during and after Ramadan. Clinical data and samples were collected at the College of Dentistry/University of Baghdad, Iraq, and the Specialized Dental Center/Al-Sadr City. Participants were assessed for periodontal parameters using the Plaque Index (PLI) and the GI. Salivary samples were collected, and pH level and salivary flow rate were determined. Enzyme-linked immunosorbent Assay was utilized to detect Glucosyltransferase and interleukin-8 levels in saliva samples.

Results: There was a non-significant rise in pH during fasting (6.09±0.77) compared to after fasting (5.80±0.78). The SFR showed a non-significant decline during fasting (0.74±0.35) when compared to after-fasting (0.88±0.43). Plaque and gingival indices exhibited non-significant changes between the two groups. Notably, GTF levels significantly decreased during fasting, while IL-8 levels showed a marked reduction during fasting (218.83±23.55 ng/L) compared to after fasting (389.07±35.93 ng/L).

Conclusions: This study found that fasting throughout Ramadan has an impact on biomarkers related to dental health. Levels of GTF and IL-8 exhibited considerable changes, whereas pH and SFR remained unchanged. Reducing GTF levels may lead to a decrease in the formation of dental plaque and tooth decay. Decreased levels of IL-8 may suggest a decrease in inflammatory activity, which in turn lowers the risk of periodontal disease.

Introduction

Ramadan, the ninth month of the lunar calendar, lasts for 29–30 days. Muslims fast from dawn until sunset. Fasting is partial, it lasts 11–18 hours of abstinence from food and drinks. Due to changing relaxation, exercise, and eating routines, Ramadan changes our daily rhythms (1). Religious fasting affects physiology and disease pathogenesis (2). Limiting food consumption may impair the immune system (3), especially when fasters ignore health risks. Oral hygiene may greatly reduce dental problems. Yet, unhealthy habits like

neglecting brushing at night, overeating, or eating high-fat meals may lead to oral disorders including dental caries, gingivitis, and periodontitis. Dental caries is one of the most frequent and complex oral health issues worldwide. Complex interactions between acid-producing bacteria, fermentable carbohydrates, host factors, and saliva are the main producers of this disease (4, 5).

Saliva maintains oral health and physiology and is a reliable biomarker detection tool for host protective components. Salivary proteins clean teeth, prevent abrasion and attrition, delay demineralization, promote remineralization, neutralize acids, and protect the oral cavity from infection (6-8).

Fasting alters immunological function, while the cellular breakdown enhances catabolism and macrophage activity (9). It improves B cell-mediated immunity, but its effects on cell-mediated immunity are unclear. Fasting increases neutrophil (bactericidal) and monocyte-killing and natural killer-cell activity (10). However, throughout Ramadan, fasting has unknown consequences on immunological responses.

Streptococcus *mutans* virulence requires GTFD to synthesize glucans on tooth and bacterial surfaces to bind streptococci and other organisms (11). *S. mutans* produces three Gtf gene products. GTFD B polymerizes a 1,3-linked glucose-rich insoluble glucan. GTFD synthesizes a soluble glucan with mostly 1, 6-linked glucosyl units, whereas GTFC synthesizes a polymer with 1,3- and 1,6-linked glucose moieties. *S. mutans*, *S. sobrinus*, and *S. sanguinus* may produce salivary GTF (12).

Glucosyltransferases are prevalent in saliva and are essential to caries development, hence may be linked to caries activity. Many diseases, start at mucosal surfaces, which are normally protected by antimicrobial proteins (13). Its salivary presence indicates the oral mucosal immune state (14).

Inflammatory cytokines including IL-1, 6 and 8 are major components of low-grade systemic inflammation that predispose to the development of different diseases such as cardiometabolic diseases, Rheumatoid arthritis, gingivitis, and periodontitis. IL-8 is a central mediator of inflammatory responses and is involved in a range of cellular activities, including cell proliferation, differentiation, and apoptosis (15).

Inflammatory and chemotactic CXC family protein IL-8 has a glutamate-leucine-arginine (ELR) motif at its N-terminus. IL-8 promotes intracellular signaling pathways downstream of CXCR-1 and CXCR-2. Monocytes, macrophages, T-cells, neutrophils, fibroblasts, endothelial cells, and chondrocytes produce IL-8 (16).
This cytokine attracts and activates the polymorphonuclear neutrophils (PMNs) on which infection, inflammation, and innate immunity depend. Oral health actually depends on neutrophil-bacterial interactions as they move from the peripheral circulation to the oral mucosal tissues towards the sites of tissue injury, inflammation, and infection to form oral polymorphonuclear neutrophils (oPMNs). Oral inflammation increases with oPMNs count (17). oPMNs function via phagocytosis, degranulation, ROS generation, and neutrophil extracellular trap (NET) formation. Reactive oxygen species change and inhibit signaling and antimicrobial molecules in the body (18-21GTFD). Therefore, the aim of this study is to understand the implications of Ramadan fasting on various dental biomarkers, such as pH, SFR, GTF, IL-8 levels in individuals with dental caries.

Subjects and Methods

A total of Forty samples including males and females in the age range of 20-25 years. This age range was chosen because it represents a young adult population that is generally healthy, but still at risk for dental issues like caries. Additionally, this age group is likely to have a stable hormonal and physiological status, reducing variability due to factors like puberty or menopause that might influence oral health. The first sample was taken during the fourth week of Ramadan fasting for individuals having dental caries. A follow-up was conducted for the same individuals two weeks after Ramadan fasting.

This study was conducted in the College of Dentistry/University of Baghdad, Iraq, and specialized dental center /Al-Sadr city. In the period between April 2023 to June 2023.

The study protocol was approved by the scientific committee at the Basic Science Department/College of Dentistry/University of Baghdad, on 1/4/2023, 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.
This longitudinal observational study summarized only participant-provided clinical data and their clinical samples and did not interfere with the patient’s therapy. Thus, this research posed no physical dangers to the participants. In addition, the confidentiality of the participants' information was ensured. The request for exemption from informed consent was submitted, and the exemption was approved.

The study focused on individuals aged 20–25 years suffering from tooth decay who took their last meal two hours before sunrise and maintained good oral hygiene. It included participants of both sex and those willing to follow up after fasting, specifically examining changes two weeks post-Ramadan fasting. Excluded were individuals with systemic, chronic, autoimmune diseases, hypersensitivity reactions, diabetes mellitus, periodontal diseases, or any inflammatory conditions. These conditions could significantly alter immune and inflammatory responses, interfere with oral health, and introduce variability that obscures the effects of fasting on oral health biomarkers. Also excluded were non-fasting subjects, individuals with structural dental defects, patients currently undergoing caries treatment, and those who smoked.

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 PLI and the GI.

The presence of plaque is a primary factor in the development of cavities (dental caries) and gingiva disease (periodontal disease). When using the PLI, 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 (8). 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 (8).

Additionally, the salivary flow rate of each subject was determined. This involved measuring the volume of saliva collected in milliliters (ml) and dividing it by the time taken for collection in minutes (min). The collection time was accurately measured using an electronic timer, and the rate of saliva secretion was expressed in milliliters per minute (ml/min):
Flow rate (ml/min) = Volume/minute (22, 23).

The pH of saliva was measured by pH meter, after calibration in buffer solution pH (every day of saliva sampling). The meter was washed with deionized water to eliminate any residues and dried. The pH meter head was entirely inserted into the cup of a saliva sample, it was left for approximately 30 seconds to gain a stable and final reading, this reading was recorded on the participant's sheet, after each reading of the saliva sample the steps of cleaning the meter and drying are started again (23).

Saliva was collected from participants at two distinct times: during Ramadan fasting and two weeks after Ramadan. For each collection, 1-3 milliliters of whole unstimulated saliva were gathered between 9 a.m. and 12 p.m. After collection, each saliva sample was placed into a sterile tube and centrifuged at 2000 revolutions per minute (rpm) for 15 minutes. This process separated the supernatant, which was then transferred to a labeled Eppendorf tube for storage. All samples were kept in a deep freezer at -80°C until they were ready for analysis.

For the analysis, the study employed the ELISA technique to detect pro-inflammatory biomarkers in the saliva samples. Specifically, it used a Human Glucosyltransferase (Catalog No: FY-EH5311, Feiyuo, China) and Human Interleukin-8 (Catalog No: FY-EH6170, Feiyuo, China) in saliva samples by ELISA reader (BioTek/USA).

The Statistical Package for Social Sciences (SPSS) version 26 and Microsoft Excel 2010 were employed for data processing. Differences between group means were studied. Fisher's exact and T. test, Wilcoxon Signed Ranks. Level of significance as: non-significant $p>0.05$, significant $p<0.05$.

Results

The study included 40 young males and females between the ages of (20-25) years. The results indicate that the mean age between females and males was non-significant different ($p>0.05$). As demonstrated in Table 1.

Table 1: Demographic data of sex and Age

Baseline Fasting Group	Sex	
	Male	Female
Statistic	No. (%)	No. (%)
	20 (50.0%)	20 (50.0%)
Fisher's Exact Test (p-value)	1.000 (0.500)	
Age (mean±SD)	22.80± 1.936	22.85±2.007
t-test (p. value)	0.006 (0.937)	

The pH and SFR levels in the study groups are shown in Figure 1. The mean value of pH showed an increase during fasting compared to after fasting (6.09±0.77, 5.80±0.78). This difference was not statistically significant in either group (p=0.112). On the other hand, the SFR level demonstrated a significant decrease in the fasting group compared to the after-fasting group (0.74±0.35 0.88±0.43) (p=0.193).

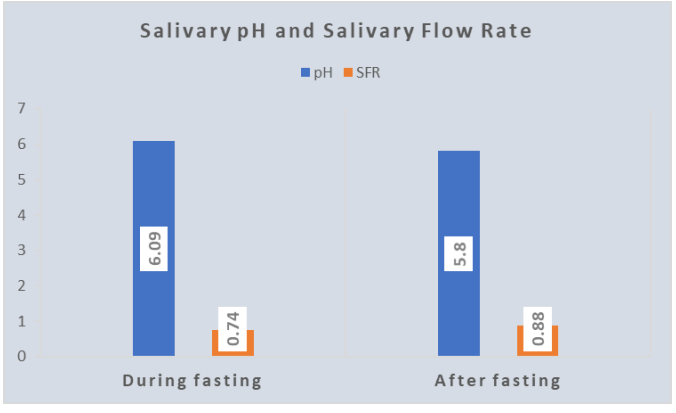

Figure 1: Levels of Salivary pH and Salivary flow rate

According to the findings of this study there is no difference ($p>0.05$), in both the Plaque Index (PI) and the Gingival Index (GI) between the two groups. The mean value for the PLI is (0.18±0.15) for the fasting group and (0.16±0.15) for the after-fasting group. Similarly, the mean value of the GI in both study groups are (0.21±0.23) and (0.24±0.11) as shown in Figure 2.

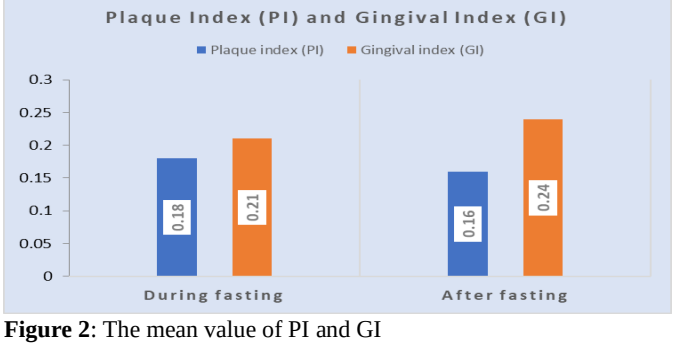

Figure 2: The mean value of PI and GI

Table 2 shows that the mean±SE value of the Glucosyltransferase enzyme decreased significantly in the fasting group compared to the non-fasting group (p=0.010).

Table 2: The alterations in GTF (ng/L) levels among individuals with dental caries, transitioning from During fasting to After fasting.

Wilcoxon Signed Ranks Test	GTF (ng/L) level	
	During fasting	After fasting
mean±SE	110.82±6.77	123.39±7.13
Z	0.267	
p-value	0.010	

The results presented in Table 3 demonstrate a decrease, in IL 8 (ng/L) levels observed in the fasting group as opposed to the non-fasting group. The mean value, during fasting was 218.83±23.55 while it was increased to 389.07±35.93 after fasting with a significant difference (p=0.01).

Table 3: The alterations in IL-8 (ng/L) levels among individuals with dental caries, transitioning from fasting to after-fasting

Wilcoxon Signed Ranks Test	IL-8 (ng/L) level	
	During fasting	After fasting
mean±SE	218.83±23.55	389.07±35.93
Z	-3.401	
p-value	0.01	

Discussion

The study included a group of 40 individuals diagnosed with caries, with a representation of both men and women. The intentional inclusion of sex distribution in this investigation is aimed at minimizing any biases related to sex. As mentioned by McGregor et al. (25), maintaining a gender-balanced cohort is crucial to account for the impact of gender differences on biomarker results, which can manifest in various ways. The mean age for both genders shows a similarity, with females having a mean age of 22.80±1.936 and males having a mean age of 22.85±2.007. This similarity in age ensures that any age-related variations are evenly distributed between the two groups. By ensuring an age distribution across each sex, the study enhances the credibility of results associated with biomarkers while considering the influence of age. However, it's important to note that when extrapolating these findings to younger cohorts, there may be some variability in the results obtained. Exploring differences between individuals and those who are middle-aged or older can provide insights into the development and manifestation of oral conditions (26). Determining the pH level in the mouth plays a role in assessing susceptibility to cavities. This is because having high pH levels is strongly associated with the loss of minerals in enamel, which eventually leads to the formation of cavities. Actually, pH value changes during fasting may be due to alterations in eating and drinking habits, which impact saliva production and composition. Saliva is crucial to maintaining oral pH. When fasting, reduced food intake can lead to decreased saliva production, altering the pH balance. Additionally, changes in the types and quantities of bacteria in the mouth during fasting can also affect pH levels (22,24). According to a research study conducted by Hurlbutt et al. (27), a pH level below 5.5 is often considered the threshold for enamel degradation. A decrease in SFR has a correlation with an increased susceptibility to dental caries. This can be attributed to reduced buffering capacities and a decreased ability to remove carbohydrates from the mouth (28).

In this study, both fasting and after-fasting groups showed low values of PI. Although there was a difference in GI values between these two groups, indicating consistent maintenance of gum health, over time this difference did not reach statistical significance (29). The fact that GTF concentrations decreased during fasting and then increased when participants resumed eating patterns after fasting demonstrates how closely nutrition and the bioactivity of GTFs are interconnected. There might be a connection between the decrease in GTF levels while fasting and the absence of its source, which is found in our diet (30). Similar to this study, Della Torre et al. (31) also discovered a drop in GTF activity during periods of food intake or fasting. However, the findings of Sheiham, A. (32) and Maniyadath et al. (33) slightly contradict this discovery. Disparities between studies may be attributable to demographic, dietary, or methodological variations. Fasting time and duration. Fasting and eating timings may dramatically alter oral health indices. The meals eaten shortly after fasting may vary by community. Others may not eat sucrose-rich meals, affecting GTF reactivation.

The present study showed low levels of IL-8 in the fasting group compared to after-fasting. The observed decrease in IL-8 levels during fasting may be a reduction in inflammation. Marsh (34) proposes a link between reduced acidity problems and decreased inflammation. Furthermore, fasting triggers changes within the body, including modulation of the immune system's response. According to Longo and Mattson (35), lower levels of markers like IL-8 may indicate that fasting has a pronounced effect on the body's physiological state. This current study aligns with Faris et al.'s research (36). In contrast, Mattson et al. (37) reported the levels of cytokines in saliva during a fasting period and found no changes in the levels of IL 8.

Limitation and 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.

Conclusion

This study found that Ramadan fasting can have distinct effects on oral health biomarkers. Although the variations in pH and saline flow rate (SFR) were not significant, substantial changes in glucosyltransferase (GTF) and interleukin-8 (IL-8) levels were observed. The decrease in GTF may potentially lead to reduced plaque formation and a lower incidence of dental caries. Similarly, a reduction in IL-8 could signify decreased inflammatory activity, which may lower the risk of periodontal diseases.

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The authors report no involvement in the research by the sponsor that could have influenced the outcome of this work.

Conflict of Interest

All the authors declare no commercial or financial conflict of interest.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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