Original Article

The Impact of Ramadan Fasting on Salivary Oxidative Stress in Relation to a Gingival Health Condition

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Abstract

Background: In the 9th month of the Islamic lunar calendar, Ramadan, Muslims fast from sunrise to sunset. Remarkably, this fasting process has been known to enhance oral and general health. **Objectives:** Male dental students undergoing Ramadan fasting were the research subjects of a study that aimed to determine the linkage between gingival health and salivary oxidative stress during the fasting. **Materials and Methods:** The subject population of this study comprised 30 male dental students aged 20–23 years old. The sample collection process entailed taking saliva from each student three times—1 week prior to Ramadan's commencement (T0), during its last week (T1), and 1 month after its end (T2). Alongside these, various clinical and periodontal evaluations were carried out. Subsequently, the samples were analyzed using ELISA for malondialdehyde (MDA)—markers of oxidative stress and a spectrophotometer for determining total antioxidant capacity (TAC). **Results:** During the course of the study, it was found that salivary MDA levels underwent a significant decrease from baseline to Ramadan ($P < 0.05$), while TAC in the saliva increased from baseline with a significant increase from baseline to after Ramadan fasting ($P < 0.05$). Significant reductions in gingival index values were observed during the baseline, Ramadan, and after Ramadan periods ($P < 0.05$). A strong positive correlation between gingival and plaque indices was observed throughout all three periods as well ($P < 0.05$). ADditionally, there was also a positive significant correlation observed between the salivary antioxidant capacity and gingival index during baseline and Ramadan ($P < 0.05$). **Conclusion:** Ramadan fasting results in lower salivary MDA levels and increased total salivary antioxidant capacity, leading to improved gingival health status.

Keywords: Gingival health, observational study, oxidative stress, Ramadan fasting, salivary antioxidant

Introduction

During Ramadan, the 9th month of the Muslim calendar of lunar months, Muslims are expected to abstain from eating or drinking between sahur and iftar.[1] This religious obligation requires all healthy adult Muslims to fast during the daytime for approximately 29–30 days. However, women who experience their menstrual cycle, children, travelers, and the ill do not have to follow this requirement.[2]

The concept of oxidative stress is based on the imbalance of reactive oxygen species (ROS) and antioxidant compounds in cells, favoring the former one leading to potential damage.[3,4] It's considered a cause for the pathogenesis of multiple diseases, including neurodegenerative diseases, heart disease, cancer, and aging.[5,6] The system of antioxidant defense is overburdened by the increased production of free radicals and reactive oxygen species.[7]

The analysis of oxidative stress biomarkers can be considered a supplemental tool for the routine diagnosis and monitoring of diseases in the oral cavity.[8]

Malondialdehyde (MDA) is the main product examined that is used to measure the peroxidation of polyunsaturated fatty acids and is a biological marker frequently used to evaluate oxidative stress. MDA is a dangerous and highly toxic compound that is suspected to have both atherogenic and mutagenic properties through its interactions with DNA and protein.[9]

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Antioxidants are all substances that inhibit or slow the process of the oxidation reaction and the chain reaction caused by free radicals; they prevent cell damage or cell death.[10] Total antioxidant capacity (TAC) is considered one of the diagnostic biomarkers of saliva, which contains both enzymatic and nonenzymatic antioxidants that act as a first line of defense against stress-induced damage to the immune system and disease of the mouth.[11]

The fasting period may have anti-inflammatory and antioxidant properties in healthy Muslims.[12] The antioxidant and reducing properties of saliva are affected by different factors, including fasting during Ramadan.[13]

Gingivitis is a condition that involves the inflammation of the tissues at the gingival margins without loss of the bone that supports the teeth.[14] A strong association exists between oxidative status and periodontal status, and they may have a significant role in the development of gingivitis and periodontitis. Oxidative stress continues to be the central part of the damage caused by the host and pathogen's interactions.[15]

The goals of the current study were to find out the Ramadan fasting effect on salivary oxidative stress of dental students in relation to gingival health status and to find the correlations between Ramadan fasting, salivary oxidative stress biomarkers, and gingival health parameters.

Materials and Methods

The participants in this observational study were 30 male dental students (College of Dentistry/University of Baghdad); their age range was from 20 to 23 years (average 21.60).

Exclusion criteria included students who had medical issues that could affect their periodontal health (such as diabetes, high blood pressure, or cardiac disease), who smoked, used supplements, or wore orthodontic or prosthetic appliances, and who had just taken medication that might affect their periodontal health.

The investigation was conducted at three different time points: the week before the start of Ramadan (T0), the last week of Ramadan (T1), and a month after the end of Ramadan (T0). Unstimulated saliva samples and clinical information regarding oral hygiene and gingival issues were evaluated during these three-time points from March to the end of May 2022.

Collection of unstimulated saliva and recoding of dental plaque and gingival health condition

According to the guidelines provided by Navazesh and Kumar in 2008,[16] the salivary sample was collected. Before collection, the participants should avoid consuming any food, gum, or beverages. Next, they should sit down and rinse their mouth with distilled water, and taking five minutes to relax. Afterward, the test tube should be placed beneath their heads, and they should lower their movement. With their mouths open, participants should let saliva flow into the tube for five minutes. At the conclusion of the collecting period, the participants were instructed to gain any residual saliva in their mouth and immediately spit it spit it into the test tube. After the foam all disappeared, the saliva was put into a cooler box and taken to the laboratory, where centrifugation must occur at around 3000 rpm for roughly 10 min. Lastly, a micropipette is used to extract the supernatant for further evaluation and stored at -20°C. Assessing oral health and hygiene among dental students was determined via two indices: Silness and Loe Plaque Index (PlI) from 1964 and Loe and Silness Gingival Index (GI) from 1963. The means of PlI and GI of all teeth for each dental student were then calculated, followed by the calculation of the mean PlI and GI of all dental students.

Biochemical analysis of salivary samples

Chemical analysis in a laboratory was used to examine salivary samples, measuring salivary antioxidant (TAC) and biomarkers of oxidative stress (MDA) concentrations calorimetrically by an automated ELISA reader (PKL PPC 230, Italy). To measure the total antioxidants of saliva with (U/mL), a colorimetric assay kit (T-AOC) from Elabscience (USA) was utilized. Meanwhile, the detection of salivary MDA levels (ng/mL) employed an assay kit from the same company, adhering to the instructions of the manufacturer.

Statistical Analysis

Utilizing the Social Sciences Package for SPSS (Version 22, Chicago, Illinois), data was described, analyzed, and presented. After undergoing the Shapiro–Wilk test, it was concluded that all examined variables were normally distributed. Significance was attributed to P values lower than 0.05. Each period of enrollment for every parameter involved in the study was subjected to separate descriptive analyses, which included means and standard deviations. Repeated measure one-way analysis of variance (ANOVA) was used to examine any potential differences in the parameters across the three separate measurement periods, which is considered a statistical test for the difference between k-related means using Bonferroni posthoc test. Significant differences between the two measurements were determined by conducting multiple comparisons between pairs of measurements from the same subject. To assess the association between clinical and laboratory values, we used Pearson's correlation. Spearman correlation was used to determine the correlation between clinical parameters. A P-value of 0.05 or less was considered significant for the statistics.

Ethical aspects

Written consent was obtained from all the participants after the Ethical Committee of the University of Baghdad's

College of Dentistry approved the research protocol. (i.e., The ethical committee authorized this study, no.486322, on January 19, 2022). Each participant was informed about the protocol's specifics before participating.

Results

The dental students who participated in the study numbered 30, all between the ages of 20 and 23. The mean age was 21.60 ± 1.133 years. The mean and standard deviation of the clinical oral hygiene and gingival parameter (plaque index and gingival index) before Ramadan fasting, at Ramadan fasting, and after 1 month of Ramadan and the results of the comparison tests for these periods are displayed in Table 1. The plaque and gingival indices' mean value continued to decline from baseline, during and after Ramadan, with a statistically significant decrease for the gingival index.

The salivary biochemical parameters mean and standard deviations before Ramadan, during Ramadan, and after a month of fasting in Ramadan and the test results comparing these three periods with each other are listed in Table 2. The table shows that MDA decreased significantly during Ramadan fasting. At the same time, there was a continuous increase in TAC during the three periods. From a statistical perspective, the difference was significant ($P < 0.05$).

The findings of the multiple pairwise comparisons test to identify any statistically significant variance between the three periods according to the one-way repeated measure (ANOVA) tests are displayed in Table 3. Consequently, for salivary measurements, there has been a significant elevation in TAC concentration from baseline to after Ramadan, and a significant decrease has been shown in MDA concentration from baseline to Ramadan ($P < 0.05$). Furthermore, for GI between periods, there was a significant difference in GI from baseline to after Ramadan and from Ramadan to after Ramadan ($P < 0.05$).

The relation between PlI and GI was demonstrated in Table 4 among dental students during the three-time interval by application of the Spearman correlation coefficient. A positive, significant, and strong association ($P < 0.05$) between PII and GI at the baseline, during fasting, and after a month of fasting.

Table 5 demonstrates the relationship between PlI and GI with salivary TAC and MDA among dental students during three times intervals. Data analysis by Application of Pearson correlation showed a negative, weak significant correlation between salivary TAC and gingival index in baseline and Ramadan periods ($P<0.05$). While for salivary MDA with PlI and GI, there was a not significant positive correlation in each period ($P > 0.05$).

Discussion

Throughout the Ramadan fast, one may observe various shifts in behavior and physicality, such as changes in their body temperature, cortisol, melatonin, and glucose

Table 1: Descriptive statistics of clinical parameters among three periods of the study

Variables		Baseline	Ramadan	After Ramadan	*F*	*P* value
PlI	Mean	0.497	0.435	0.415	2.358	0.113
	±SD	0.286	0.308	0.223		
GI	Mean	0.294	0.206	0.129	12.629	**0.000**
	±SD	0.302	0.225	0.149		

Bold value indicates significance $p < 0.05$

Table 2: Descriptive statistics for salivary total antioxidant (TAC) and salivary malondialdehyde (MDA) among periods

Variables		Baseline	Ramadan	After Ramadan	*F*	*P* value
MDA (ng/mL)	Mean	159.112	92.751	113.633	3.856	**0.033**
	±SD	131.240	39.502	78.103		
TAC (U/mL)	Mean	14.262	15.348	17.773	3.877	**0.033**
	±SD	5.423	4.919	6.974		

Bold value indicates significance $p < 0.05$

Table 3: The multiple-pairwise comparisons for gingival index and salivary parameters by the repeated measure one-way ANOVA test

Variables	Baseline-Ramadan		Baseline-after Ramadan		Ramadan-after Ramadan	
	Mean difference	*P* value	Mean difference	*P* value	Mean difference	*P* value
GI	0.088	0.064	0.166	**0.000**	0.078	**0.005**
TAC (U/mL)	-1.089	0.921	-3.511	**0.025**	-2.425	0.192
MDA (ng/mL)	66.361	**0.026**	45.479	0.087	-20.882	0.501

Bold value indicates significance $p < 0.05$

Table 4: Correlations between gingival index with plaque index by time periods

Variables	Baseline GI		Ramadan GI		After Ramadan GI	
	rsp	*P* value	rsp	*P* value	rsp	*P* value
PlI	0.632	**0.000**	0.606	**0.000**	0.689	**0.000**

Bold value indicates significance $p < 0.05$

Table 5: Correlation of plaque and gingival indices with salivary biomarkers by three periods

Variables		TAC (U/mL)		MDA (ng/mL)	
		R	*P*	*r*	*P*
Baseline	PlI	-0.247	0.189	0.122	0.521
	GI	-0.430	**0.018**	0.229	0.224
Ramadan	PlI	-0.283	0.130	0.221	0.240
	GI	-0.409	**0.025**	0.131	0.491
After	PlI	-0.303	0.104	0.069	0.717
	GI	-0.330	0.075	0.226	0.230

Bold value indicates significance $p < 0.05$

regulation. The circadian rhythm's distribution may also be impacted. ADditionally, sleepiness and daytime awareness may be subject to alteration, as well as nocturnal routines. These physiological changes can impact the immune system and oxidative stress levels within the body.[17] Saliva, being comprised of similar components as blood, is considered a reflection of both an individual's general health and disease-related properties.[18]

As revealed by our latest research, the concentration of salivary MDA (which is a sort of lipid peroxidation) was found to be significantly reduced throughout Ramadan's fasting. This can be ascribed to a decline in the output of mitochondrial reactive oxygen species. As a result of fasting during Ramadan, the number of calories and nutrients declines, which can prevent the deterioration of lipids, proteins, and DNA. Hence, cell damage and oxidative damage are minimized.[19,20] Previous studies have corroborated the same findings.[21-23] While other investigations reported that during the fast of Ramadan, the MDA level in blood serum had been found to remain unchanged.[24,25] It is important to note that the previous studies conducted were limited to blood serum.

This study also found that dental students experienced a consistent rise in their saliva's TAC throughout the post-Ramadan period compared to the baseline, with a significant difference. The boost in TAC levels can be credited to several factors, including the activation of the Krebs cycle during fasting. This, in turn, leads to the generation of intermediate molecules that serve as electron donors to antioxidant substances in saliva and the body,[26] oxidation status was under control, and DNA damage was reduced by the elevation of TAC. Another explanation for TAC elevation is that the antioxidants are necessary to inhibit the oxidation reaction produced in the oral cavity, thereby reducing the free radicals.[27] This is supported by the current study's results, which demonstrated a decrease in MDA, which is indicative of a negative correlation between markers of MDA and TAC. ADditionally, the increase in TAC following the Ramadan fast was attributed to the resumption of typical eating patterns and increased consumption of fruits and vegetables; this led to an increase in antioxidants, which enhanced the mechanism of defense against excess ROS.[28,29] The same findings were reported in another investigation.[30] Additionally, a different study reported an increase in serum TAC during Ramadan's fasting,[31] while another study reported no alteration in serum TAC during Ramadan's fasting.[32]

The results of this investigation also demonstrated a decrease in the gingival index, which may be attributed to a decrease in dental plaque, considered the most significant cause of gingivitis.[33] The number of bacteria decreases by the decrease of plaque index, and the toxin of bacteria also decreases, leading to diminish in the gingival disease.[34] There was a positive correlation between the plaque and the gingival indices,[35-37] which is supported by the result of this study. The significant drop in the gingival index may also be caused by a decrease in the amount of reactive oxygen species; this was manifested in the study as a decrease in the salivary level of MDA, a biomarker of oxidative stress that is, involved in the development and progression of numerous periodontal diseases.[38] Another possible explanation for the decrease in the gingival index could be due to increasing TAC concentration within saliva which has an anti-inflammatory effect on the immune system; this effect decreases the expression of inflammatory molecules in the immune system's fighters, such as monocytes in the gingival connective tissues.[39] This is supported by this study that found an increase in the concentration of TAC from the baseline to the after Ramadan period and a significant negative correlation between the index and the concentration of TAC before and during the Ramadan

period. Another potential explanation is that Ramadan fasting decreases the inflammation process by decreasing leukocytes and decreasing the release of proinflammatory cytokines (IL-1β, IL-6, and TNF-α).[12] The same outcome was observed in one other study that was not a human study,[40] and the opposite was reported in another study.[41]

Conclusion

Ramadan fasting has positive effects on gingival health. During Ramadan fasting, salivary oxidative stress was significantly reduced, and salivary antioxidant capacity was significantly increased, thereby improving gingival health by reducing gingivitis during this month. To enhance the effect of fasting during Ramadan month on oral and gum health, additional research with larger samples and other salivary parameters is required.

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Conflicts of interest

There are no conflicts of interest.

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