

# Ramadan Fasting in Relation to Salivary Uric Acid and Selective Physical Parameters

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## Abstract

**Background:** Ramadan is the holy month in which all Muslims abstain from eating and drinking from sunrise to sunset. The impact of Ramadan fasting on the oral cavity and saliva needs more attention because of the important function of saliva in the protection of the mouth.

**Objective:** To determine the impact of Ramadan fasting on salivary uric acid, flow rate, and pH among Iraqi male dental students.

**Patients and Methods:** The subject population consisted of thirty healthy male dental students (College of Dentistry/University of Baghdad) with an age range of 20–23 years who intended to fast the whole of Ramadan (1443 Hijri calendar). Saliva samples were obtained from all dental students one week prior to the start of Ramadan, the last week of Ramadan, and one month after the end of Ramadan. The salivary flow rate was calculated, and the salivary pH was measured using a digital pH meter. Then a saliva sample was analyzed to detect the concentration of uric acid by using the spectrophotometer.

**Results:** The data analysis for this study revealed that the salivary flow rate decreased from baseline to Ramadan and then increased again after one month of Ramadan with significant differences ( $p < 0.05$ ). While salivary uric acid increased continuously with significant differences from baseline to Ramadan and after one month of Ramadan fasting ( $p < 0.05$ ), on the other hand, salivary pH showed no significant differences ( $p > 0.05$ ) among the three time periods of the study.

**Conclusion:** Saliva was affected by Ramadan fasting through a decrease in salivary flow rate during fasting and a return to normal salivary flow rate after the end of Ramadan. In addition, uric acid, which acts as a salivary antioxidant, shows an increase during Ramadan fasting.

**Keywords:** Ramadan fasting, salivary flow rate, salivary pH, uric acid

## Introduction

Ramadan is the ninth month of the Islamic lunar calendar, which has great significance for all Muslims globally. This time period is characterized by abstinence from food and drink as well as from sexual activity. Muslims fast daily from sunrise to sunset throughout the month of Ramadan [1]. The oral cavity reveals a change in eating

patterns, timing, and dental cleanliness throughout the holy month of Ramadan; as a result of this variation, the microflora in the mouth can change [2].

Saliva is a bodily fluid produced by salivary glands that mostly comprises water, proteins, glycoproteins, electrolytes, tiny organic molecules, and substances, all of

which are transferred from blood circulation [3].

The major salivary gland produces 90% of saliva, whereas the minor salivary glands create 10% of saliva in addition to several constituents, such as gingival crevicular fluid (GCF), desquamated epithelial cells, debris of food, bacteria and their secretions, serum and blood derivatives, viruses and fungi, and other cellular components [4].

In the oral cavity, saliva serves a variety of purposes, including speech, mastication, gustatory sensitivity, deglutition, mucosal invasion protection, tissue lubrication, antibacterial, antiviral, and antifungal activity, ionic balance regulation at enamel remineralization, post-eruptive maturation, acid diffusion restriction, and deposition of acquired enamel pellicle [5].

Due to its nature, content, and functions, as well as linkages with other bodily systems and structures, saliva laboratory analysis has become an essential tool for the assessment of physiological and pathological states. In comparison to blood testing, saliva testing has several advantages such as painless sample, simplicity of storage, and cheap cost of analysis [6].

Several factors, including salivary flow rate, buffering capacity, pH, and consistency, can be used to evaluate saliva [7]. Changes in salivary secretion's quantity and/or quality may have negative consequences on the mouth (caries, oral mucositis, candidiasis, oral infections, chewing problems), as well as the extraoral (dysphagia, halitosis, weight loss) [8-9].

The unstimulated salivary flow rates of healthy individuals have found the average value for whole saliva to be about 0.3 ml/min

[10]. Numerous factors, including gender, nausea, fasting, the circadian and circannual cycles, the size of the salivary gland, body weight, pressure, radiation, smoking, systemic hydration, body posture, nutrition, systemic disease, and medicines, all had an impact on salivary flow rate [11]. The term pH is used to describe the chemical acidity and alkalinity levels of a substance [12]. The normal range of salivary pH is 6.4–7.4 [13]. salivary flow rate and salivary pH are considered part of the defense mechanisms of saliva alongside salivary buffer capacity, electrolytes, and the antioxidant system [14]. In saliva, uric acid serves as the most significant antioxidant, accounting for 85% of the antioxidant activity [15]. The uric acid concentration in the saliva is equal to its concentration in serum [16]. Furthermore, it is an important salivary biomarker with clinical importance in monitoring oxidative stress and metabolic syndrome [17] and has the capacity to chelate transition metals as well as interact with biological oxidants such as reactive nitrogen species, hypochlorous acid, and hydroxyl radicals [18]. Also, uric acid has an important effect on the health of the oral cavity [19]. So antioxidants have an important effect on oral health status [20, 21]. The current study was conducted to determine the effect of Ramadan fasting on salivary uric acid, flow rate, and pH among Iraqi male dental students.

## **Patients and Methods**

The total sample for this observational case series study was 30 male dental students (College of Dentistry / University of Baghdad) with an age range between (20-23) years old (mean age 21.60). After explaining the study's goal and methodology to each

participant verbally and in writing, they each provided written informed consent to take part in this follow-up study. The exclusion criteria include dental students with any systemic disease such as diabetes mellitus, hypertension, or cardiovascular disease that may affect oral health conditions, those who smoked, used dietary supplements or wear orthodontic or prosthetic appliances, as well as those who had recently taken medications that could have an impact on periodontal health conditions or had a course of anti-inflammatory or antibiotic treatments during the last month before examinations. The current study was conducted over three-time points in the period from march to the end of May 2022: one week before the beginning of Ramadan (1443 Hijri calendar), the final week of Ramadan month, and one month following the end of Ramadan fasting month, unstimulated salivary samples were assessed during these three-time points. the baseline of the study was taken one week before fasting.

### **Study of salivary secretion**

The investigation of salivary secretion was carried out without any external stimulus between nine and eleven in the morning, under normal temperature and humidity conditions. According to the guidelines provided by Navazesh and Kumar (2008) [22], Before saliva was collected, all subjects remained for at least an hour without eating or drinking. On a chair, they should sit, rinse their mouth with distilled water, and relax for at least five minutes. Then subjects should reduce their movement and be instructed to fix their foreheads above and the test tube beneath them. After that, the subjects were told to keep their mouths open so that saliva

could drip into the tube for five minutes. They were instructed to collect any residual saliva in their mouth and immediately spit it into the test tube at the end of the collecting period. After the foam had all disappeared, the salivary flow rate was calculated by dividing the milliliters (ml) of collected saliva by the minutes needed for collection (min). Only 5 minutes were used as a collection time measured by an electronic timer. The pH of saliva was measured by a digital pH meter (checked by Hannah). The head of the pH meter was entirely sunken into the cup of a saliva sample and left for approximately 30 seconds inside to gain a stable and final reading. The saliva was put into a cooler box and taken to the laboratory, where it was centrifuged at 3000 rpm (revolution per minute) for 10 minutes. The supernatant was then separated using a micropipette and stored at  $-20^{\circ}\text{C}$  for further analysis to determine the uric acid concentration.

The level of salivary uric acid was determined calorimetrically using the automatic ELISA reader (PKL PPC 230, Italia). With the use of a uric acid colorimetric assay kit (Elabscience, USA). Uric acid content can be calculated by measuring the optical density value at 690 nm. The gingival health condition was assessed using the gingival index, according to Loe and Silness (1963).

### **Statistical Analysis**

The Statistical Package for the Social Sciences (SPSS version 22, Chicago, Illinois, USA) was used for data description, analysis, and presentation. All studied variables were normally distributed among periods using the Shapiro-Wilk test at  $p > 0.05$ . For each

period of registration and for each parameter used in this study, descriptive statistical analyses of variables, including means and standard deviations, were conducted independently. Repeated Measure One Way (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 the Bonferroni posthoc test. Following these analyses, a multiple pairwise comparisons test was utilized to find any statistically significant differences between two measurements taken from the same subject. By using Pearson correlation, the relationships between clinical and laboratory parameters were analyzed. Statistics were considered significant at P values < 0.05. By using G Power version 3.1.9.7, statistical power analyses were conducted.

## Results

A total of 30 dentistry students, whose ages ranged from 20 to 23 years, have a mean age of 21.60 ±1.133 years. Table (1) shows the mean values and standard deviations of salivary flow rate among dental students during three-time intervals. From the table, it was found that the salivary flow rate decreased in Ramadan and then increased again after Ramadan, ending with significant differences (p<0.05). The results of the one-way repeated measure (ANOVA) test using multiple pairwise comparisons to determine whether there was a statistically significant difference between the three periods are shown in Table (2). From the table, it was found that there was a significant difference (p<0.05) in salivary flow rate from baseline to Ramadan and from Ramadan to after Ramadan.

**Table (1):** Salivary flow rate during the three periods of the study

Variables	Salivary flow rate(ml/min)			
	mean	SD	F	p-value
Baseline	0.547	0.194	15.507	<b>0.000*</b>
Ramadan	0.413	0.163		
After Ramadan	0.550	0.233		

\*=significant at p<0.05, SD=standard deviation, ml/min=millimeter/minute

**Table (2):** Multiple Pairwise Comparisons of salivary flow rate among periods using Bonferonni posthoc test

Variable	Salivary flow rate (ml/min)	
	MD	P value
Baseline- Ramadan	0.133	<b>0.000*</b>
Baseline-after Ramadan	-0.003	1.000
Ramadan-after Ramadan	-0.137	<b>0.007*</b>

\*=significant at p<0.05, MD=mean difference, ml/min= milliliter /minute

Table (3) demonstrates the mean value and standard deviation of salivary pH among dental students during three time points. From the table, it was found that salivary pH

decreased from baseline to Ramadan and then increased again after one month of Ramadan, ending with no significant difference (p > 0.05) among the three periods of the study.

**Table (3):** Salivary PH during the three periods of study

Variables	Salivary pH			
	Mean	SD	F-value	p-value
Baseline	6.847	0.198	1.401	0.263*
Ramadan	6.793	0.205		
After Ramadan	6.820	0.207		

\*symbol denotes not significant at  $p > 0.05$ , SD: standard deviation

Table (4) demonstrates the mean and standard deviation of salivary uric acid for dental students at three-time intervals. From the table, it was found that there was a continuous increase in UA levels from the baseline until 30 days after the end of Ramadan fasting, with a significant difference ( $p < 0.05$ ). Table (5) demonstrates the multiple pairwise comparisons for the results among periods of the study. It was found that there was a significant increase in

UA concentration from baseline to Ramadan and after Ramadan ( $p < 0.05$ ), while the concentration of UA from Ramadan to after Ramadan was not significant ( $p > 0.05$ ).

Table (6) demonstrates the negative relationship between salivary uric acid concentration and gingival index during the three periods of the study by using Pearson's correlation. Statistically, all the correlations in this table were not significant ( $p > 0.05$ ).

**Table (4):** Salivary uric acid during three periods of the study

Variables	UA (mg/L)			
	mean	SD	F-value	p-value
Baseline	80.543	29.092	14.539	<b>0.000*</b>
Ramadan	101.179	23.404		
After Ramadan	106.201	25.654		

\*significant at  $p < 0.05$ , SD=standard deviation, UA =uric acid, mg/L= milligram per

**Table (5):** Multiple Pairwise Comparisons of salivary uric acid among periods using Bonferonni posthoc test

Variable	UA (mg/L)	
	MD	p-value
Baseline- Ramadan	-20.636	<b>0.000*</b>
Baseline- after Ramadan	-25.658	<b>0.000*</b>
Ramadan -after Ramadan	-5.022	0.489

\*significant at  $p < 0.05$ , MD: mean difference, UA: uric acid, and mg/L: milligram per liter

**Table (6):** Correlation coefficient between gingival health condition and salivary uric acid among periods by using Pearson’s correlation

periods	Variable	UA (mg/L)	
		r	p
Baseline	GI	- 0.124	0.513
Ramadan	GI	- 0.329	0.075
After Ramadan	GI	- 0.056	0.768

\*GI: Gingival index, UA :uric acid, and mg/L: milligram per liter

## Discussion

In the current study, unstimulated saliva was collected because unstimulated saliva protects the oral tissue by keeping the oral cavity moist and lubricating the oral mucosa at all times [23, 24]. Also, another study revealed that salivary stimulation alters the amount of saliva, the concentration of particular constituents, and the pH of the fluid [25].

In the present study, results showed that there was a significant decrease in salivary flow rate among dental students from baseline to Ramadan. This result could be attributed to the fact that the decrease in salivary gland stimulation is caused by a lack of gustatory stimulation. The autonomic nervous system regulates the secretion of different salivary components as well as the salivary flow rate [26]. Salivary secretions and salivary flow rate change in response to autonomic nervous system stimulation [27]. During Ramadan fasting, sedentary activity with minimal orofacial movement and metabolism slows down the function of tissue cells in the body, including the cells in the oral cavity, which may explain the low stimulation of the autonomic nervous system [28]. That led to a decrease in the salivary flow rate. Another explanation for this decrease could be attributed to the dehydration associated with fasting [29],

which will lead to a decreased salivary flow rate [30].

The same result was found in other studies in which [31] reported that salivary flow rate was significantly decreased by fasting when compared to a non-fasting person. Moreover, [32] reported that the salivary flow rate ranged from 0.08 to 1.40 ml/min at rest and showed about a 10% decrease in response to fasting when compared to the non-fasting period [31, 32].

While another study by reported no change in the salivary flow rate during Ramadan and 3 weeks after it [33]. On the other hand, this study found that the significant elevation in salivary flow rate after one month of Ramadan could be attributed to the return to a normal routine of eating and drinking after Ramadan, which led to increased mastication and gustation that stimulated salivary flow rate [34]. Another study also reported the same result [33]. Additionally, the result of the current study shows a non-significant decrease in the acidity of the saliva from baseline to Ramadan, with a slight increase in the acidity from Ramadan to after Ramadan. The same result of increased pH in saliva during Ramadan fasting was reported by [35]. reported an increase in the acidity of saliva during Ramadan fasting [28]. However, the above-mentioned result shows significant differences. Furthermore, in the

present study, the salivary concentration of uric acid was continuously increased from baseline until 30 days after the end of Ramadan, with a significant difference from baseline to Ramadan and after Ramadan ends. Elevating salivary UA levels could be attributed to some probable reasons; first, it could be due to the fact that the body breaks down stored proteins, amino acids, and lipids for energy during the fasting days of Ramadan; uric acid is a byproduct of this catabolic process [36], which in turn elevates uric acid concentration during fasting. Second, it could be attributed to the decrease in the salivary flow rate, which results in increased salivary protein concentration [37], and this is supported by the result of the current study, which revealed a decrease in the salivary flow rate during Ramadan fasting. The third explanation for uric acid elevation during Ramadan fasting is that Ramadan fasting had a possible anti-inflammatory and antioxidative effect on healthy Muslims [38], and uric acid makes up 70–85% of the entire antioxidative capacity of saliva [39]. The same result was also reported by Ooi et al. (2019), in which serum uric acid was significantly increased during the fasting period [40]. On the other hand, other studies on saliva reported the opposite result, in which [28] reported that salivary uric acid was significantly decreased during Ramadan fasting. And [41] reported a significant reduction in the level of salivary uric acid for the fasting group when compared with the non-fasting control group [28,41]. While another study by Oueslati reported that serum uric acid was not significantly changed during Ramadan fasting [42].

## Conclusions

The findings of the present study demonstrate that saliva was affected by Ramadan fasting through a decrease in salivary flow rate during fasting and a return to normal salivary flow rate after the end of Ramadan. Also, uric acid, which acts as a salivary antioxidant, shows an increase during Ramadan fasting that provides protection for the oral cavity during the fasting period.

## Recommendations

It is recommended to increase the intake of water and healthy foods after breaking the fast with an increase in oral care practices such as dental brushing, flossing, and the use of meswak during the holy month.

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**Ethical clearance:** The study had been approved by the ethical committee at the College of Dentistry/University of Baghdad. (i.e., the ethical committee authorized this study, No. 486322).

**Conflict of interest:** Nil

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## صيام رمضان وعلاقته بحمض اليوريا اللعابي وبعض الصفات اللعابية المختارة ضمن مجموعة من طلبة كلية طب الاسنان العراقيين

ريام عبد الكريم حسين<sup>١</sup>, بيداء حسين عون<sup>٢</sup>

### الملخص

**خلفية الدراسة:** شهر رمضان المبارك هو أحد الشهور الهجرية، إذ يتمتع المسلمون البالغون جميعاً في أنحاء العالم كله عن الطعام والشراب منذ شروق الشمس إلى الغروب. للصيام تأثير كبير على صحة الفم واللحسان بصورة خاصة وذلك لأهمية اللعاب في حماية الأنسجة الفموية.

**اهداف الدراسة:** لتقييم تأثير الصيام على مستوى تدفق اللعاب، حموضة اللعاب ومستوى حمض اليوريا اللعابي ضمن مجموعة من طلبة طب الأسنان الذكور فقط .

**المرضى والطرائق :** يشمل الموضوع ٣٠ طالباً من كلية طب الأسنان /جامعة بغداد تتراوح أعمارهم بين ٢٠ - ٢٣ سنة لمن أرادوا صيام رمضان ١٤٤٣ للهجرة. تم جمع عينات اللعاب غير المحفزة من الأفراد جميعاً قبل أسبوع من بداية شهر رمضان، والأسبوع الأخير من شهر رمضان، وبعد شهر من انتهاء شهر رمضان. تم قياس مستوى تدفق وحموضة اللعاب ثم القيام بعمل مختبري كيميائي حيوي لقياس مستوى حمض اليوريا اللعابي للعينات ضمن فترات الدراسة الثلاثة.

**النتائج:** أظهرت النتائج انخفاضاً في مستوى تدفق اللعاب للفترة من قبل رمضان الى رمضان ثم زيادة في مستوى تدفق اللعاب للفترة من رمضان الى بعد رمضان مع وجود فرق ذو دلالة إحصائية  $p < 0.05$ . بينما أظهرت النتائج وجود زيادة مستمرة ذات دلالة إحصائية  $p < 0.05$  لحمض اليوريا اللعابي قبل وخلال وبعد رمضان . من ناحية أخرى أظهرت النتائج انخفاضاً في مستوى حموضة اللعاب مع عدم وجود فرق ذو دلالة إحصائية خلال الفترات الثلاث من الدراسة.

**الاستنتاجات:** ان صيام رمضان يؤثر على اللعاب من خلال انخفاض مستوى تدفق اللعاب في الفم خلال فترة الصيام وعودة مستوى تدفق اللعاب الى الزيادة بعد انتهاء فترة الصيام. كذلك للصيام تأثير على مستوى حمض اليوريا اللعابي الذي يعتبر جزء من مضادات الاكسدة اللعابية حيث سجلت زيادة في تركيز حمض اليوريا خلال فترة الصيام لتوفير الحماية لأنسجة الفم.

**الكلمات المفتاحية:** صيام رمضان، معدل تدفق اللعاب، درجة الحموضة اللعابية، حمض اليوريا

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