# Immunodulatory Effects of Ramadan Fasting

### By

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# يوخته:

ئامانج : ئامانجى ئەم توێژينەوە بريتى يە لە ھەڵسەنگاندنى كاريگەرى بەڕۆژووبون لەسەر ھەندى سايتۆسىنەكانى ھەوكردن و دژە ھەوكردن وە لەسەر ئاستى سروشتى بۆ گلۆبىنەكانى بەرگرى .

ئەنجامەكان : بەرزبونەوە لە سايتۆكىنەكانى دژە ھەوكردن IL و IL و IL ووى داوە , وە دابەزىنى سايتۆسىنەكانى IL IFN- $\alpha$  , IFN- $\alpha$  , IFN- $\gamma$  , IFN- $\gamma$  , IFN- $\alpha$  , IFN- $\alpha$  , IFN- $\gamma$  , IFN- $\alpha$  , IFN- $\gamma$  , IFN- $\alpha$  , IFN- $\alpha$  , IFN- $\gamma$  , IFN- $\gamma$  , IFN- $\alpha$  , IFN- $\alpha$  , IFN- $\gamma$  , IFN- $\gamma$  , II- $\alpha$  , II- $\alpha$ 

دەرئەنجامەكان : رۆژووگرتنى مانگى رەمەزان لەوانەيە ھەلئكى زۆر باش بدات بە دەستەوە بۆ سوك كردنى ھەندى لە نەخۆشىيە ھەوكاريەكان .

راسپاردهكان : ئەنجامدانى تويزينەوەى زياتر لەسەر ئەوانەى كە توشى نەخۆشىيە ھەوكاريەكان بوون وەك نەخۆشى بەرگرىيە خۆييەكان .

### الخلاصة:

الهدف: الهدف من الدراسة الحالية هو تقييم تأثير صيام شهر رمضان على بعض الحركيات الخلوية (الإلتهابية /الضد إلتهابية) و على المستويات الطبيعية للكلوبيولينات المناعية.

المن هجىء: جُمعت عينات الدم قبل الصوم كمجموعة ضابطة أولى وبعد (14, 28) يوم مِنْ الصيام لأربعين شخص جميعهم ذكور, غير متزوجين,غير مدخنين,أصحاء ظاهريا وتتراوح أعمارهم ما بين (20-30) سنة, في شهر رمضان (2012). وتم قياس مستويات الحركيات الخلوية (IL-4, IL-17A, TGF-β, TNF-α, IFN-γ) بتقنية ELISA ومستويات الكلوبيولينات المناعية (IgA, ) بطريقة (IgG, IgM)

النتائج: كان هناك إرتفاع في الحركيات الخلوية الضد إلتهابية 4-I و IL-4 وانخفاض في الحركيات الخلوية الإلتهابية IL-17 وغير معنوية في IL-17 وكانت الفروقات معنوية (P <0.05) في حالة IL-17 , IR- $\alpha$  ,

الأستنتاجات: إن صيام شهر رمضان قد يعطي فرصة ممتازة للتَحسين عدد من الأمراض الإلتهابية. التوصى ات: إجراء المزىد من الاستنتاجات: إن صيام شهر رمضان قد يعطي فرصة ممتازة للتَحسين عدد من الأمراض الألتهابية مثل مرض المناعة الذاتية.

## **Abstract**

**Objective:** To assess the effect of Ramadan fasting on some immunological parameters, including cytokines (Inflammatory /anti-inflammatory) and Immunoglobulins levels.

**Methods:** Blood samples were collected before fasting as a control group and after (14, 28) days of the fasting. These tests were done on sample of forty apparently healthy males who were singles; non-smoker and their age range were 20-30 years in the month of Ramadan (2012). Enzyme-linked Immune Sorbent Assay (ELISA) technique was used to measure cytokines levels before and during fasting while Single Radial Immuno Diffusion (SRID) was used to measure immunoglobulin levels.

**Results:** There was elevation in the levels of anti-inflammatory cytokines IL-4 and TGF- $\beta$  and a decreasing in inflammatory cytokines TNF- $\alpha$ , IFN- $\gamma$ , IL-17A cytokine level and the differences were significance in case of TNF- $\alpha$ , IFN- $\gamma$ , IL-4, TGF- $\beta$  (P>0.05) but not significant in case of IL-17A. The results showed that, there was a significant decrease (P<0.05) in the serum IgA level and not significant a decrease in IgG levels during fasting. The study showed that, IgM level was not affected by fasting.

**Conclusions:** Ramadan fasting provides an excellent opportunity to ameliorate a number of the auto inflammatory diseases.

**Recommendations:** Performing more studies on subjects affected with inflammatory disease such as autoimmune disease.

Key words: Ramadan fasting, TNF- $\alpha$ , IFN- $\gamma$ , IL-4, IL-17A and TGF- $\beta$ , IgA,IgG,IgM.

#### Introduction:

Ramadan fasting has been shown to modulate certain aspects of the immune system <sup>[1]</sup>. Although the immunoregulatory effects of low-caloric diets on various components of the immune system have been

demonstrated  $^{[2]}$ , the effects of Ramadan fasting on immune system function have not yet been adequately characterized. Ramadan fasting can lead to some beneficial changes in some inflammatory markers. The effect of fasting on the immune system and the relationship between the immune system and metabolism are important scientific and practical problem due to a wide use of low-caloric diets $^{[3-7]}$ . Experimental fasting data demonstrate the important role of cytokine during fasting which manifested by their influence on the endocrine system $^{[8,9]}$ . Several studies have demonstrated the effects of religious fasting on physiology and disease pathology. Although limiting the total food intake could potentially weaken the immune system, it is usual for an individual who is fasting during Ramadan to have a balanced eating, resting and sleeping schedule. Together, this should help to maintain adequate immune function  $^{[1, 2, 3, 5]}$ . The aim of this study was to evaluate the effect of Ramadan fasting on some cytokines namely TNF- $\alpha$ , IFN- $\gamma$ , IL-4, IL-17A and TGF- $\beta$  and levels of Immunoglobulins (IgA, IgG, IgM).

# **Subjects and methods**

# **Subjects**

Forty apparently healthy single males at 20-30 years of age, who indicated that they were going to fast during Ramadan during 2012, were recruited to the study. Women were excluded from the study since they are prohibited to fast during their menstrual cycle due to religious rules. In addition, subjects with any acute or chronic diseases, or those who used medications during the study period, were excluded. The content of the subjects' diets was similar before and during Ramadan.

### **Methods**

Blood samples (5ml from each participant) were collected in plain tubes. Sera were separated by low-speed centrifugation at 1000 g for 15 min at room temperature. Samples were immediately separated into aliquots and stored at (-20 C) until analyzed. All serum samples

were analyzed in a single batch to avoid day-to-day laboratory variations. A sandwich type (ELISA) was used to measure serum levels of (IL-4, IL-17A, TGF- $\beta$ ,TNF- $\alpha$ , IFN- $\gamma$ ) concentrations. In addition,

concentrations of serum levels IgG, IgA and IgM were determined by using Single Radial Immuno Diffusion (SRID) method to measure immunoglobulins.

## Statistical analysis

Statistical analysis was done using a one way of analysis of variance (ANOVA). For all analyses, a value of (P<0.05) was considered significant. All statistical analyses were performed statistical Package for Social Science (SPSS) V20.

#### Results

Changes in inflammatory cytokines (TNF- $\alpha$ , IFN- $\gamma$ , IL-17A) during Ramadan are given in Table (1, 2, 3) respectively. TNF- $\alpha$  and IFN- $\gamma$  decreased significantly (P<0.05) during Ramadan compared with before fasting while IL-7A was not significantly differed. The results of anti-inflammatory cytokines IL-4 and TGF- $\beta$  are given in Table (4, 5). IL-4 and TGF- $\beta$  increased significantly (P<0.05). Changes in serum Ig concentrations during Ramadan are shown in Table (6-8). IgA concentrations were significantly (P<0.05) decreased during Ramadan compared with before fasting (Table 6) ,and serum IgG level also decreased but not significantly Table (7). These changes in immunoglobulin levels were still within the normal range; however, there was no significant change in serum IgM levels (Table 8).

**Table (1)** showed the mean $\pm$  S. E of TNF- $\alpha$  level (pg/dl) in normal healthy objects of prefasting in comparison with means of fasting period.

		N	Mean Std. Error±	F	Sig
TNF-α	Pre-fasting	40	12.288 <sup>a</sup> 0.6274		0.000
	After(14)days	40	9.978 <sup>b</sup> 0.5039	19.309	
	After(28)days	40	7.698 <sup>c</sup> 0.4132		

- Similar letters mean non-significantly different (P>0.05).
- Non-Similar letters mean significantly different (P<0.05).

**Table (2)** showed the mean± S. E of IFN-γ level (pg/dl) in normal healthy objects of prefasting in comparison with means of fasting period.

		N	Mean Std. Error±	F	Sig
Υ _	Pre-fasting	40	48.223 <sup>a</sup> 4.0577		0.000
	After(14)days	lays 40	38.880 <sup>ab</sup> 3.0947	9.632	
	After(28)days	40	28.668 <sup>b</sup> 1.9376		

- Similar letters mean non-significantly different (P>0.05).
- Non-Similar letters mean significantly different (P<0.05).</li>

**Table (3)** showed the mean± S. E of IL-17A level (pg/dl) in normal healthy objects of prefasting in comparison with means of fasting period.

		N	Mean Std. Error±	F	Sig
	Pre-fasting	40	37.52 <sup>a</sup> 3.708	1.773	0.174
IL-	After(14)days	40	31.69 <sup>a</sup> 3.143		
7A	After(28)days	40	28.99 <sup>a</sup> 2.921		

- Similar letters mean non-significantly different (P>0.05).
- Non-Similar letters mean significantly different (P<0.05).

**Table (4)** showed the mean± S. E of IL-4 level (pg/dl) in normal healthy objects of prefasting in comparison with means of fasting period.

		N	Mean± Std. Error	F	Sig
	Pre-fasting	40	13.323ª .5364		
	After(14)days 40 After(28)days 40		18.808 <sup>b</sup> .6839	48.257	0.000
			24.100° 1.0247		

- Similar letters mean non-significantly different (P>0.05).
- Non-Similar letters mean significantly different (P<0.05).

**Table (5)** showed the mean $\pm$  S. E of TGF- $\beta$  level (pg/dl) in normal healthy objects of prefasting in comparison with means of fasting period.

		N	Mean± Std.Error	F	Sig
TGF- β	Pre-fasting	40	48.80 <sup>a</sup> 4.350		
	After(14)days	40	54.70 <sup>ab</sup> 3.848	2.946	0.005
	After(28)days	40	62.15 <sup>b</sup> 3.440		

- Similar letters mean non-significantly different (P>0.05).
- Non-Similar letters mean significantly different (P<0.05).</li>

**Table (6)** showed the mean± S. E of IgA level (mg/dl) in normal healthy objects of prefasting in comparison with means of fasting period.

		Ν	Mean ± Std. Error	F	Sig
	Pre-fasting	40	189.693 <sup>a</sup> 18.4171		
IgA	After(14)days 40 After(28)days 40		107.260 <sup>b</sup> 7.8933	27.345	0.000
			67.668 <sup>b</sup> 4.8662		

- Similar letters mean non-significantly different (P>0.05).
- Non-Similar letters mean significantly different (P<0.05).</li>

**Table (7)** showed the mean± S. E of IgG level (mg/dl) in normal healthy objects of prefasting in comparison with means of fasting period.

		N	Mean± Std. Error	F	Sig
Pre-fasting		40	1878.63 <sup>a</sup>		
	1 10 1001119		170.389	2.041	0.135
	After(14)days	40	1792.15 <sup>a</sup>		
IgG			137.611		
	After(28)days	40	1501.08 <sup>a</sup>		
			97.722		

- Similar letters mean non-significantly different (P>0.05).
- Non-Similar letters mean significantly different (P<0.05).</li>

**Table (8)** showed the mean± S. E of IgM level (mg/dl) in normal healthy objects of prefasting in comparison with means of fasting period.

		N	Mean± Std. Error	F	Sig
IgM	Pre-fasting	Pre-fasting 40 255.705 <sup>a</sup> 20.9655			
	After(14)days	40	258.150 <sup>a</sup> 20.8447	0.036	0.965
	After(28)days	40	263.425 <sup>a</sup> 20.4363		

- Similar letters mean non-significantly different (P>0.05).
- Non-Similar letters mean significantly different (P<0.05).</li>

### **Discussion:**

The effect of Ramadan fasting in inflammatory cytokines was assessed via blood analysis of various inflammatory / anti-inflammatory cytokines. In addition to standard immunoglobulins. The elevated serum levels of anti-inflammatory cytokines (IL-4, TGF- $\beta$ ) and lowered inflammatory cytokines (TNF- $\alpha$ , IFN- $\gamma$ , IL-17A) may contribute to an imbalance between inflammatory and anti-inflammatory cytokines in favor of the later. Data presented in this study are differentially congruous to data presented by other studies [1,2,3,5,10,11,12]. It can be said that many factors can play role; including spiritual status and the season which Ramadan month falling in. Still the majority of the studies dealt with this area of research denote the beneficial effects of this manner of fasting [1,12,13,14], if compared to a very little studies coming with incongruous finding. In conclusion, the results denoted that moreover it is safe, Ramadan fasting may provide an excellent opportunity to ameliorate a number of the auto inflammatory disease.

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