

# Evaluation of the Effects of Ramadan Fasting on Lymphocyte subpopulations in a Two-year Follow-up

Zahra Siadat<sup>1</sup>, Maryam Rastin<sup>2</sup>, Nafise Tabasi<sup>2</sup>, Abdolrahim Rezaee<sup>2</sup>, Mahmoud Mahmoudi<sup>2\*</sup>

<sup>1</sup> Department of Nutrition, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

<sup>2</sup> Immunology Research Center, Department of Immunology and Allergy, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

## ARTICLE INFO

*Article type:*  
Original article

*Article History:*  
Received: 26 Dec 2013  
Revised: 01 Feb 2014  
Accepted: 23 Jan 2014  
Published: 11 Mar 2014

*Keywords:*  
Lymphocyte subpopulations  
Ramadan fasting  
Immune system

## ABSTRACT

Today, the interactions of the immune system, nutrition, and nervous system are one of the main research areas of interest in immunology and disease treatment. Due to changes in the mood, behavior, and diet of an individual during fasting period, the body's internal homeostasis is affected. The aim of the present study was to evaluate the effects of Ramadan fasting on lymphocyte subgroups, which are the main specific immune cells in the body. For this purpose, in years 1999 and 2000, thirty-eight healthy Muslims (9 females and 29 males), within the age range of 17 to 51 years (mean age=35.4 years), were assessed before the start and one day before the end of Ramadan.

The pre-Lymphocytic subpopulations analysis was conducted using flow cytometry. The results showed that the percentage of total lymphocytes was 25.82% and 26.23% in the pre- and late-Ramadan periods, respectively; the observed difference was insignificant. However, the absolute lymphocyte counts were  $2.3 \times 10^3$  and  $2.1 \times 10^3$  mm<sup>3</sup> before and late Ramadan, respectively, and the difference was considered significant ( $P$ -value=0.06).

The percentage of CD3<sup>+</sup> cells (T cells) was 70.12% before Ramadan and 70.25% late Ramadan, and the absolute lymphocyte counts were  $1.6 \times 10^3$  and  $1.5 \times 10^3$  mm<sup>3</sup>, respectively; therefore, the differences were not significant. Regarding the subgroups of CD4<sup>+</sup> cells (T<sub>H</sub>), the percentage ratios of the cells were 53.46% and 52.8% in the pre- and late Ramadan periods, and the absolute counts were  $0.087 \times 10^3$  and  $0.081 \times 10^3$  mm<sup>3</sup>, respectively; however, the differences were not significant in this cell subgroup.

The percentage of CD8<sup>+</sup> (T<sub>C</sub>) cells was 37.7% before Ramadan and 37.8% late Ramadan, and the absolute counts were  $0.6 \times 10^3$  and  $0.54 \times 10^3$  mm<sup>3</sup> in the pre- and late-Ramadan periods, respectively; therefore, the differences were considered insignificant. In addition, the percentage ratios of B-lymphocytes cells were 14.56 % and 14.74% in the pre- and late-Ramadan periods, and the absolute count changed from  $0.35 \times 10^3$  to  $0.3 \times 10^3$  mm<sup>3</sup>. According to the results, the differences were not significant, therefore, it seems Ramadan fasting does not affect these cells.

Moreover, the percentage of activated T cells or T<sub>DR</sub><sup>+</sup>, which are involved in specific immune responses, has not been affected by fasting. In fact, the percentage ratios were reported as 11.14% and 10.54% in the pre- and late-Ramadan periods, and the absolute count changed from  $0.14 \times 10^3$  to  $0.11 \times 10^3$  mm<sup>3</sup>; the differences were not considered significant.

Finally, the ratio of CD4<sup>+</sup>/CD8<sup>+</sup> cells or T<sub>H</sub>/T<sub>C</sub> changed from 1.48% before Ramadan to 1.5% late this month; however, this difference was insignificant. Thus, the overall results indicate that Ramadan fasting during winter does not affect the lymphocyte count, percentage ratio, and the main lymphocyte subpopulations.

► Please cite this paper as:

Siadat Z, Rastin M, Tabasi N, Rezaee A, Mahmoudi M. Evaluation of the Effects of Ramadan Fasting on Lymphocyte subpopulations in a Two-year Follow-up. J Fasting Health. 2014; 2(1): 31-36.

## Introduction

In recent years, the scientific methodology of detail-based studies, or the evaluation of molecular components without considering the holistic review of the results, has been a matter of great controversy. Thus, more attention has

been paid to the relationship between molecules, cells, and systems, and their interaction effects on one another.

The immune system with its set of cells and molecules plays an essential role in maintaining

\* Corresponding author: Mahmoud Mahmoudi, Immunology Research Center, Department of Immunology and Allergy, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. Email: mahmoudim@mums.ac.ir

© 2014 mums.ac.ir All rights reserved.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

internal homeostasis (1, 2). However, immune system cells and cytokines are essential to form a healthy appropriate microchemical environment. Therefore, in recent years, a new field of study called "psychoneuroimmunology" has emerged with the aim to evaluate the mechanisms of forming appropriate microchemical environments for mental, psychological, and physical health (3-7).

On the other hand, a great deal of attention has been focused on nutrition, essential ingredients for energy intake, necessary substances for the body, and rare elements affecting the coenzyme and enzyme reactions and their resultant effects on the immune system. For instance, the role of calorie intake in cancer-related complications, and the importance and effects of trace elements (especially zinc and selenium) on the immune system balance and protection against autoimmune diseases and cancer, have gained increasing interest (8-10).

According to Muslims' beliefs, Ramadan fasting is a behavioral, mental, and dietary change, which is significantly different from food deprivation and malnutrition (11). Many studies have been performed on food deprivation (fasting) or the effects of malnutrition and special diets on lymphocyte subpopulations (11-13).

Most of these studies are concerned with fasting as the treatment of some diseases such as immune system complications (including autoimmune diseases). However, the fasting conditions of these studies were not similar to Muslims' religious rules. In one of these studies, it was shown that after 7 days of complete food deprivation, the microchemical environment of the white pulp of the spleen, especially the T-dependent zone, experienced more significant reducing lymphocytes. Food deprivation even caused inhibitory effects on the specific immune system. However, non-specific immunity (innate immunity), particularly the activity of natural killer cells (NK cells), which is necessary for anti-cancer and anti-viral defense, increased (11).

In another study, the subjects fasted for seven full days by only water and electrolyte intake. According to the results, the level of lymphocyte count, the number of helper cells

( $T_H$ ), and the production of IL-2 in response to PHA stimulation, reduced (13). In a complete three-to-six day food deprivation of patients with rheumatoid arthritis and healthy subjects, the systemic immune response did not greatly alter, though specific mucosa-derived B-lymphocyte response increased (14, 15).

In Islamic countries, the evaluation of the effects of fasting is mostly focused on biochemical factors, and white blood cell differential count (16-19); nevertheless, some studies were conducted on specific subjects (such as cancer patients and transplant recipients). For instance, a study performed on renal transplant patients, showed that fasting had no side effects on the renal activity or patients' health status. Moreover, no negative effects were observed in cancer patients who insisted on fasting, in case they followed the medical advice regarding their situation (20).

In this study, we aimed to evaluate the effects of Ramadan fasting on the main components of specific immune system, including lymphocytes and their main subpopulations in a two-year follow-up.

## Materials and Method

In the present study, 38 healthy subjects (9 females and 29 males), within the age range of 17 to 51 years, were selected; the subjects had no history of serious health conditions in the past five years. The participants were monitored in two consecutive years of 1999 and 2000 during the month of Ramadan, which fell in winter.

Ramadan fasting in this study was defined as abstinence from eating, drinking, and other behaviors, which have been mentioned in Islamic rules (at least 22 days of fasting during the 30 days of Ramadan). Fasting duration in the aforementioned years was estimated approximately 12 to 12.5 hours.

The subjects were selected before the month of Ramadan, and the sampling was carried out two days before the start and one day before the end of Ramadan. Two ml of venous blood was obtained from each individual, and transferred to BuAli Research Institute, Mashhad, Iran for testing. Flow-cytometry was immediately started using the flow cytometer (BD, USA).

In this study, the labeled antibodies against

**Table 1.** The percentage ratio and absolute count of peripheral blood lymphocytes before and late Ramadan fasting

Cell type	Absolute count ( $10^3/\text{mm}^3$ )		Percentage ratio (%)		Results
	Pre-fasting period	Late-fasting period	Pre-fasting period	Late-fasting period	Statistical test
Total lymphocytes	2.32±0.4	2.19±0.5	25.82±8.2	26.23±7.3	<i>P</i> = 0.06

**Table 2.** Comparison of the percentage ratio and absolute count of T and B lymphocytes before and late Ramadan fasting

Cell type	Absolute count ( $10^3/\text{mm}^3$ )		Percentage ratio (%)		Results
	Pre-fasting period	Late-fasting period	Pre-fasting period	Late-fasting period	Statistical test
T cells (CD3 <sup>+</sup> )	1.61±0.35	1.5±0.4	70.12±5.9	70.26±6.4	*
B cells (CD19 <sup>+</sup> )	0.35±0.09	0.3±0.09	14.56±3.24	14.74±3.7	*

\*Non-significant

CD3, CD4, CD8, CD19, HLA-DR were used. After the flow cytometry results were recorded, the results related to each person were obtained and recorded in a specific form, based on the percentage ratio and absolute count. A questionnaire was also completed by the subjects in order to collect demographic data. After gathering the data, they were analyzed using descriptive statistics by drawing tables and graphs. The research hypotheses were tested using the statistical tests of Wilcoxon signed-rank, paired t-test, and matched-paired t-test, based on a normal distribution.

## Results

In the present study, which was conducted on 38 subjects (9 females and 29 males, with the mean age of 32.5 years), the mentioned parameters before and late Ramadan fasting were analyzed.

The percentage of CD3<sup>+</sup> cells (T cells) was 70.12% before Ramadan and 70.25% late Ramadan, and the absolute lymphocyte counts were  $1.6 \times 10^3$  and  $1.5 \times 10^3$   $\text{mm}^3$ , respectively; however, the observed differences were insignificant. Therefore, the percentage ratio and the absolute count of lymphocytes were not affected by Ramadan fasting (Table 1).

According to Table 2, considering the subgroups of CD3<sup>+</sup> lymphocytes or T lymphocytes, the percentage ratio and absolute count of these lymphocytes have insignificantly changed after one month of fasting (Table 2).

Also, regarding B lymphocytes, which are responsible for specific humoral immunity in the body, the changes of percentage ratio and absolute count of these cells before and late Ramadan fasting were non-significant. Therefore, Ramadan fasting has no effects on B lymphocyte count.

T lymphocyte subgroups play an essential role in specific immunity, especially T helper cells, which are central to humoral and cellular immune responses. According to the results, the changes of CD3<sup>+</sup> and CD4<sup>+</sup> lymphocyte subgroups or T helper cells (or T<sub>H</sub>) were insignificant. Consequently, Ramadan fasting has no effects on this important subgroup of T lymphocytes (Table 3).

In addition, regarding the subgroup of killer T-cells, which plays a role in the destruction of infected and cancerous cells, the results indicate that the changes in the percentage and absolute count are scarce, and no significant differences were observed after fasting (Table 3). The percentage ratio at the time of sampling and the absolute count ( $\text{mm}^3$ ) of the activated T cells after fasting had not significantly changed (Table 3).

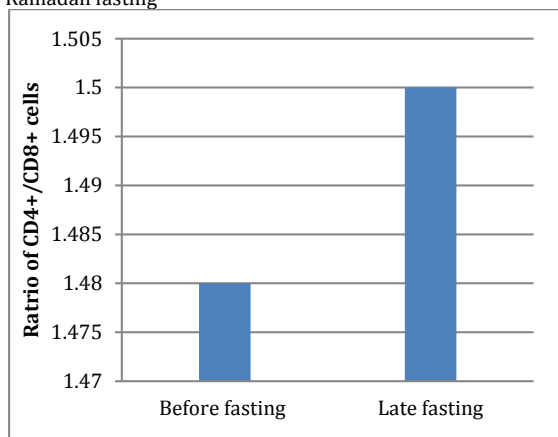
The main aim of the present study was the comparison of CD4<sup>+</sup>/CD8<sup>+</sup> cells ratio (or T<sub>H</sub>/T<sub>C</sub> cells ratio), which is an important indicator of the activity of the immune system. This ratio was 1.48% and 1.5% in the pre- and late-fasting periods; however, the difference was not statistically significant (Figure 1). Moreover, this

**Table 3.** Comparison of the percentage ratio and absolute count of T helper cells (or TH), activated T cells and killer T-cells before and late Ramadan fasting

Cell type	Absolute count ( $10^3/\text{mm}^3$ )		Percentage ratio (%)		Results
	Pre-fasting period	Late-fasting period	Pre-fasting period	Late-fasting period	Statistical test
T <sub>H</sub> (CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>+</sup> )	52.8±8.9	53.46±8.2	0.81±0.29	0.87±0.24	*
T <sub>C</sub> (CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>+</sup> )	37.8±6.7	37.7±7.8	0.54±0.16	0.60±0.18	*
T CD3 <sup>+</sup> HLA-DR	10.51±4.8	11.14±8.2	0.11±0.05	0.14±0.07	*

\*Non-significant

**Figure 1.** Comparison of CD4<sup>+</sup>/CD8<sup>+</sup> cells before and late Ramadan fasting



ratio is separately demonstrated for males and females.

## Discussion

The effects of nutrition and dietary habits on the immune system have been a subject of investigation for many years. Even the association between starvation, drought, and the outbreak of infectious diseases has been known for a long time (21). The considerable reduction in the essential nutrients for the body led to an understanding of the effects of food deprivation on the immune system. Therefore, today, it is believed that the most important cause of immune deficiency is poor diet and inaccessibility of medications (21, 22).

In addition, in recent years, the roles of antioxidants (particularly vitamins A and E) and rare elements (especially selenium) have been determined in immune-related diseases. For instance, lack of vitamin A causes a reduction in the ratio of CD4<sup>+</sup>/CD8<sup>+</sup> cells or decreases CD28<sup>+</sup> expression. The complex relationship between lipids rich in unsaturated fatty acids and the immune system, and the effects of these lipids on lymphocyte subgroups or cytokine production are also of high significance.

Ramadan fasting cannot be defined as a poor diet or reduced amount of food consumption. In fact, it is a type of nutritional change in the dietary patterns and meals, and its effect should be differentiated from food deprivation. Moreover, fasting is an alteration in circadian rhythm and daily routines, therefore, it will

mostly affect the body through the neuroendocrine system.

Various studies in Western countries on volunteers, who abstained from eating for several days, show that food avoidance has different effects on the immune system or autoimmune diseases. The results of other studies have shown decreased lymphocyte count, decreased production of interferon gamma, and no changes in serum cytokine level (12-15). For instance, long-term food deprivation for seven consecutive days (with only mineral and water intake) resulted in a decrease in the size of spleen white, particularly the T-dependent area. On the contrary, the number of natural killer cells, which are the first line of defense against viral and tumor activities, increased (11, 23).

In our study, the absolute blood leucocyte count decreased, which was statistically significant at the level of 94% (p value=0.06). However, CD3<sup>+</sup> cells (T cells), CD19<sup>+</sup> cells (B Cells), T<sub>H</sub> cells (CD3<sup>+</sup> cells), CD4<sup>+</sup> CD8<sup>+</sup>, and T<sub>C</sub> cells (CD3<sup>+</sup>, CD4<sup>+</sup>, and CD8<sup>+</sup>) did not considerably change after Ramadan fasting. Hansen (13) believes that the reduction in white blood cell count is not due to its decreased production in the bone marrow, but is related to cells migration and the tendency for implantation in the non-vascular space, particularly migration of neutrophils and implantation of lymphocytes.

However, in the present study, the slight insignificant increase in the ratio of CD4<sup>+</sup>/CD8<sup>+</sup> cells is noteworthy; the mean of the pre-fasting period (1.48) increased to 1.5 in the late-fasting period. The current study did not pay particular attention to the role of religious behaviors in the individual. In future studies considering this factor as the main variable, a better dynamic balance may be observed in the immune system. However, in the study of Savehdahl (13), which was conducted on 10 volunteers during a complete 7-day food deprivation (with only water and mineral intake), the number of CD3<sup>+</sup> (T cells) and TCD4<sup>+</sup> cells showed a significant reduction. In addition, IL-2 production decreased in response to mitogen in the culture environment.

Such studies have been performed in order to evaluate the treatment of rheumatoid

arthritis and other diseases. For instance, Trollmo (15), in a study on 7 patients with rheumatoid arthritis during a complete 3-7 day food deprivation, showed that the response of mucosal B lymphocytes increased, though the systematic responses did not alter. However, in the study of Fraser (14), on 9 patients with rheumatoid arthritis during 7 days of food deprivation (with a diet of mineral water) indicated a reduction in free fatty acid concentration, which inhibits lymphocyte proliferation responses in the fasting group with rheumatoid arthritis (15).

One of the theories with regard to the occurrence of rheumatoid factor (RF) is abnormal glycosylation of IgG, which is believed to be involved in the pathogenesis of rheumatoid arthritis. A study on 26 patients with rheumatoid arthritis, who fasted for 7-10 days and followed a vegetarian diet 3.5 months after the experiment, showed a high ratio of non-abnormal glycosylation of IgG, compared with the control group. This was associated with the patient's health improvement during the fasting period and unrelated to the vegetarian diet (24).

Finally, consistent with our study, it was shown that fasting has no negative effects on the function of transplanted kidney in kidney transplant recipients, who fasted during Ramadan (25). It should be noted that the present study was conducted in December and November of 1999 and 2000, respectively (Ramadan fell in winter). It is suggested that the current study be repeated during the Ramadan of other years, which falls in summer season (with a longer fasting duration).

## References

- Blalock JE. The immune system as a sensory organ. *J Immunol.* 1984;132(3):1067-70.
- Pawelec G, Adibzadeh M, Pohla H, Schaudt K. Immunosenescence: again of the immune system. *Immunol Today.* 1995;16(9):420-2.
- Blalock JE. The syntax of immune-neuroendocrine communication. *Immunol Today.* 1994; 15(11): 504-11.
- Brines A. Neuroendocrinoimmuno-logytoday. *Immunol Today.* 1996;15(11):503.
- Guidi L, Tricerri A, Frasca D, Vangeli M, Errani AR, Bartoloni C. Pychoneuroimmunology and aging. *Gerontology.*1998;44(5)247-61.
- Moynihan JA, Kruszewska B, Brenner GJ, Cohen N. Neural, endocrine, and immune system interactions. Relevance for health and disease. *Adv Exp Med Biol.* 1998;438:541-9.
- Muller N, Ackenheil M. Psychoneuroimmunology and the cytokine action in the CNS: implications for psychiatric disorders. *Prog Neuropsychopharmacol Biol Psychiatry.* 1998; 22(1): 1-33.
- Beck MA. Selenium and host defence towards viruses. *Proc Nutr Soc.* 1999;58(3):707-11.
- Chandra RK. Nutrition and immunology: from the clinic to cellular biology and back again. *Proc Nutr Soc.* 1999;58(3):681-3.
- Harbige Ls. Nutrition and immunity with emphasis on infection and autoimmune disease. *Nutr Health.* 1996;10(4):285-312.
- Kuziv Oie. The effect of complete fasting on the structural - functional organization of the white pulp of the spleen. *Fiziol Zh.* 1997;43(1-2):89-100.
- de Swart RL, Ross PS, Vos JG, Osterhaus AD. Short term fasting does not aggravate immunosuppression in harbor seals (*Phoca vitulina*) with high body burdens of organochlorines. *Environ Health Perspect.* 1996; 104 Suppl 4:823-8.
- Savendahl L, Underwood LE. Decrease interleukin-2 production from cultured peripheral blood mononuclear cells in human acute starvation. *J Clin Endocrinol Metab.* 1997;82(4):1177-80.
- Fraster DA, Theon J, Rustan AC, Forre O, Kjeldsen-Krag J. Changes in plasma free fatty acid concentrations in rheumatoid arthritis patients during fasting and their affects upon T-lymphocyte proliferation. *Rheumatology.* 1999; 38(10): 948-52.
- Trollmo C, Verdrengh M, Tarkowski A. Fasting enhances mucosal antigen specific B cell responses in arthritis. *Ann Rheum Dis.* 1997; 56(2):130-4.
- Witte-Foster SR, Garcia PA, Dove CR. Lipid profiles, serum immunoglobulins, dietary intake, and drug use of order rural Iowa women. *J Am Diet Assoc.* 1991;91(6):679-85.
- Aybak M, Turkoglu A, Sermet A, Denli O. Effect of Ramadan fasting on platelet aggregation in healthy male subjects. *Eur J Appl Physiol Occup Physiol.* 1996;73(6):552-6.
- Husain R, Cheah SH, Duncan MT. Cardiovascular reactivity in Malay Moslems during Ramadan. *Singapore Med J.* 1996;37(4):398-401.
- Nomani MZ, Hallak MH, Siddiqui IP. Effects of Ramadan fasting on plasma uric acid and body weight in healthy man. *J Am Diet Assoc.* 1990; 90(10):1435-6.
- Celik I, Barista I, Firat D. Cancer therapy during Ramadan. *J Natl Cancer Inst.* 1996;19;88(12):838.

21. Harbige LS. Nutrition and immunity. *Immunology News*. 1997;4(4):219-21.
22. Abbas AK, Lichtman AH, Pober JS. *Cellular and molecular immunology*. 4<sup>th</sup> ed. Philadelphia: WB Saunders Co; 2000: 466-7.
23. Hansen K, Sickelmann F, Pietrowsky R, Fehmn HL, Born J. Systemic immune changes following meal intake in humans. *Am J Physiol*. 1997; 273(2 Pt 2):R548-53.
24. Kjeldsen-Kragh J, Sumar N, Bodman-Smith K, Brostoff J. Changes in glycosylation of IgG during fasting in patients with rheumatoid arthritis. *Br J Rheumatol*. 1996; 35(2):117-9.
25. Abdalla AH, Shaheen FA, Rassoul Z, Owda AK, Popovich WF, Mousa DH, et al. Effect of Ramadan fasting on Moslem kidney transplant recipients. *Am J Nephrol*. 1998;18(2):101-4.