The Effect of Ramadan Fasting on Tuberculin Skin Test and Leukocyte Count

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INTRODUCTION

Ramadan is a holy month, when millions of Muslims around the world abstain from eating and drinking from dawn to dusk. This practice induces changes in the diet and sleep pattern of the individuals and has significant medical and physiological effects on their body (1). During the month of Ramadan fasting (i.e., a type of intermittent fasting), some changes occur in the cytokines and circadian rhythms of hormones.

Regarding this, the evaluation of leukocyte count and tuberculin skin test, which are closely related to immune system, can render interesting and helpful results (2, 3, 4).

There are few, if any, studies investigating the aspects of immune system, especially the tuberculin skin test, in Ramadan. Therefore, the present study aimed to assess the leukocyte count and tuberculin skin test during the Ramadan fasting to provide the physicians with a broader view over the impacts of the Ramadan fasting on these variables and improve their interpretation in this regard.

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Tuberculosis (TB) is a globally prevalent infectious disease that annually infects and kills millions of people around the world (5). Tuberculin purified protein derivative (PPD) is currently the most available skin test for the diagnosis of TB. This test, which highly depends on the delayed-type hypersensitivity and cellular immune response, checks for either current infection with Mycobacterium or history of TB vaccination (6). Consequently, any condition that alters the body immunity, such as rheumatoid arthritis or immunosuppressive agents, may change the PPD reaction (7).

Some studies have observed no changes in the circulating immune complexes after Ramadan fasting (8). However, other studies have reported decreased immunoglobulin G (within normal range) and increased lymphocytes (9), along with slight changes in the mean level of cytokines (interleukins) (10-17). Regarding the proposed effects of Ramadan fasting on the body immunity and the dependence of PPD to the cellular immunity, investigation of possible alteration of PPD during or after Ramadan is essential. Nevertheless, few studies have investigated the impacts of Ramadan fasting on the alteration of PPD response (13). As a result, we aimed to investigate the possible variations of PPD test during and after Ramadan fasting.

Material and methods

Study design

A total of 42 males who fasted during one month of Ramadan were included in the present study using the convenience sampling method. Out of the included participants, 14 subjects were excluded from the study due to infection with chickenpox-herpes zoster- (one case) and not referring for the follow-up after Ramadan (13 cases). As a result, this study was conducted on 28 participants during October 2006-January 2007.

For ensuring that the participants will hold onto fasting, they were selected from the seminary students of Borujen in Chaharmahal and Bakhtiari, Iran. The healthy seminary students who fasted during the whole month of Ramadan and were willing to participate in the study were included in the study. On the other hand, the students who had acute viral and autoimmune diseases or used immunosuppressant drugs, and those who discontinued the protocol of the study were excluded from the study.

The tuberculin test was performed for all the participants, and their blood samples were collected at the fourth week of Ramadan and three months after Ramadan. For the purpose of the study, 2 ml of venous blood sample was taken from all the subjects in a sitting position from the medial cubital vein on the left hand in the morning. The blood samples were kept in citrated tubes including ethylenediaminetetraacetic acid and immediately sent to the laboratory. Subsequently, the samples were analyzed for white blood cells (WBC) count using Colter device (Sysmex KX21, made in Japan).

For tuberculin test, the anterior surface of the forearm was prepared with alcohol swab; then, 5 units (0.1 ml) of PPD solution was injected intradermally. The injection was performed using an insulin syringe (1/2 inch, 27 gauge). The test was read at 72 h, and the diameter of skin induration was measured in millimeters in the transverse dimension (i.e., perpendicular to the long axis of the forearm). The tuberculin test was considered positive when the skin reaction was greater than 10 mm.

Ethical considerations

The protocol of the present study was approved by the Shahrekord University of Medical Sciences. The design and objectives of the study were explained to all participants and the written informed consent was obtained from those who were willing to participate in the study. In addition, they were clarified about the possibility to leave the study whenever they wished to.

Statistical analysis

For the quantitative variables, the results were presented as mean and standard deviation. Additionally, the continuous variables were compared using paired t-test, and Wilcoxon signed-rank test. Tuberculin test positivity was compared by McNemar’s test. The data analysis was performed though the SPSS version 11.5 (SPSS Inc., Chicago, IL). P-values of 0.05 or less were considered statistically significant.

Results

According to the results of the study, the mean age of the participants was 19.2±3.8 years (age range: 14-35 years). Furthermore, the mean tuberculin induration was 9.2±5.4 mm (size range: 2-22 mm) at the fourth week of...
Ramadan, which increased insignificantly to 9.79±6.8 mm (size range: 3-35 mm) three months after this period (P=0.501) (Table 1). In addition, the tuberculin test was positive in 39.3% and 32.1% of the participants at the fourth week of Ramadan and three months after Ramadan (P=0.774), respectively. Totally, 13 (46.4%) and 15 (53.6%) participants had a maximum of 3 mm and more than 3 mm induration change in the tuberculin test.

The mean of WBC count decreased from 5907±1879 mcL (range: 3400-11800 mcL) to 5601±1362 mcL (range: 3600-9200 mcL) after Ramadan; however, it was not statistically significant (P=0.334). Additionally, the mean lymphocytes count significantly decreased from 2292±520 mcL (range: 1200-3400 mcL) to 2023±486 mcL (range: 1200-3200 mcL) after Ramadan (P=0.003) (Table 1). Likewise, the mean of lymphocyte and neutrophil percentages reduced from 40.69±9.57% to 36.3±6.45% (P=0.014) and 51.03±10.56% to 61.17±6.17% (P<0.001), respectively (Table 1). Furthermore, no association was indicated between the PPD test and WBC count, lymphocyte, or neutrophil count (P>0.05).

**Discussion**

The results of the present study indicated a significant decrease in the lymphocyte and neutrophil count and percentage after Ramadan, compared to the last week of Ramadan. However, no relationship was indicated between these variables and the PPD results. In addition, the changes in the PPD results was not statistically significant.

In a study carried out by Rahmani and Zarei, PPD variation (before and after Ramadan) was compared between 25 non-fasting control students and 36 male students who had the same age range as our participants. They observed a significant increase in the PPD induration in the fasting group (18), which is inconsistent with the results of the present study. This discrepancy between the findings might be due to the employment of different periods for PPD measurement.

Furthermore, in the present study, we measured PPD at the end of Ramadan with a three-month interval to minimize the booster phenomenon, while in the aforementioned study, the PPD was measured just before and after Ramadan, and a control group was used to minimize the booster phenomenon. Another reason for this disagreement can be ascribed to the possibility that the results of the mentioned study were affected by some factors as the researchers did not specify how they were able to find a Muslim control group who did not fast without medical conditions, they also did not indicate whether all indurations were read by the same person.

**TB is one of the most important infectious diseases that is still common in today’s world and annually kills millions of people around the world (10).** The PPD test is a sensitive and specific test, which is considered the gold standard diagnostic tool. Nevertheless, the positivity of the skin reaction depends on several factors, including patient’s age, lifestyle, and medical or environmental risk factors (14). Accordingly, the reports about the results of this test might be biased in some studies. To eliminate the inter-observer bias, the health staff who reads the induration must be well trained (15). Regarding this, in the current study, all the PPD tests were performed by one professional to minimize this possible bias. In addition, the delayed-type hypersensitivity that forms the induration is affected by the immunologic characteristics of the participants. Therefore, we excluded all the immunosuppressed participants to minimize such bias in the current study. Consequently, the comparison between studies can be only performed in case of the similarity between the study conditions. Therefore, it is necessary that further studies evaluate the effect of Ramadan on PPD, and metaanalysis studies review this issue...
since there are few studies in this regard.

Another objective of the present study was the investigation of WBC changes due to Ramadan fasting. There are contradictory findings in terms of the effect of Ramadan on leukocyte count in the literature. Consistent with the present study, some studies have indicated no significant change in the WBC count after Ramadan fasting in spite of the identification of small changes in other serum parameters (15, 16, 17). On the other hand, some studies have reported increased leukocytes, like a study conducted by Nematy et al. investigating the effect of Ramadan fasting on several serum parameters in 82 male and female patients with coronary artery disease (18).

Likewise, Askari et al. compared a fasting asthmatic group with a non-fasting control group and reported increased monocyte counts (19). However, Chaouachi et al. revealed insignificant changes in the leukocyte cell count (i.e., neutrophils and lymphocytes) before, during, and after Ramadan despite observing significant changes in other parameters, such as C-reactive protein, haptoglobin, immunoglobulin A, and thyroid hormones that indicate alterations in body immunity due to Ramadan fasting (11).

Furthermore, Faris et al. reported decreased total leukocyte count and lymphocyte after Ramadan, compared to before this period (12). It should be mentioned that WBC can easily be affected by minor infections or inflammations, and this issue might have caused the discrepancies between the results of these studies. Moreover, these studies might not be easily comparable due to the different characteristics of the participants, including the underlying immunologic status, physical activity, and economic status. Additionally, the fact that the meals, which fasting people eat within a study cannot be controlled may also play a role in the inconsistency between the findings of these studies.

The current study included several strengths, including controlling the interobserver bias by using the same expert for examining and reporting the PPD tests in all participants. In addition, the three month follow-up minimized the possible booster phenomenon of PPD. Furthermore, we tried to select the participants from the seminary students in order to minimize the confounding effect of economic status, physical activity, and diet.

Nevertheless, the results of the present study might be affected by some limitations. Firstly, the results could not be compared with a matched control group since it was not possible to find a group of students without medical conditions who did not fast (as Ramadan fasting is a religious obligation for all Muslims). Secondly, the sample size of the present study was relatively small. Thirdly, the present study only included male participants, which limited the generalizability of results to general population. Regarding this, future multicentric studies are recommended to consider these factors using a larger sample size to determine the pure effect of Ramadan fasting on PPD and leukocyte count.

Conclusion
As the findings of the present study indicated, Ramadan fasting does not alter PPD results; however, it can significantly decrease the lymphocyte and neutrophil percentage and count.

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