CIRCULATING IMMUNE COMPLEX DURING RAMADAN

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Background: Fasting during Ramadan represent one of the five pillars of the Islamic religion. So it is important to know the effect of this kind of fasting on people's life especially on their health. The purpose of this study was to show the effect of fasting during Ramadan on circulating immune complex (CIC) level and immune system. **Methods**: The CIC levels were measured before and after Ramadan by polyethylenglycole method. The blood samples were examined using quantitation chemiluminesence and circulating immune techniques. The results were analyzed using t-test and calculated coefficient correlation between CIC before and after Ramadan. **Results**: There was no significant difference between the CIC level before and after Ramadan. **Conclusion:** According to the results, fasting during Ramadan does not have bad effect on immune system of healthy people.

Keywords: CIC; Ramadan; Fasting; Immune complex

INTRODUCTION

Ramadan occurs in the ninth month of the lunar calendar, lasting between 29 and 30 days. The lunar calendar does not correspond to the Geregorian calendar; therefore, Ramadan's occurrence can vary from one season to another.

Daily routines are markedly altered during Ramadan. They also vary depending on geographic situation, socioeconomic level and specific customs of each country.[1] Thus the obligation to eat only during the night leads to a definite change in the rhythm of life, sleep, eating schedule and the alternation of rest and activity are specially affected.[1]

Fasting during Ramadan represent one of the five pillars of the Islamic religion. So it is important to know the effect of this kind of fasting on people's life especially on their health. These days people pay a lot to protect themselves against diseases and to reach this goal they should have an intact immunity system. So we tried to find the effect of one of important events of muslim's life (Ramadan) on their health in general and immune system in specific.

During last decades, several studies have shown the effects of religions fasting on physiology or disease such as pregnancy [2] hyperlipidemia [3] and also renal allograph.[4] Fasting effects on biochemical and hematology parameters have also been subjects of intense investigations.[3]

In other studies no statistically significant changes were seen in mean body weight, total cholesterol level or LDL level but the mean HDL cholesterol level increased significantly during Ramadan.[5] Others found that eating behavior during Ramadan may contribute to improve nutritional status of people who are at risk of nutritional deficiency.[6] Another study showed the Ramadan fasting in patients with well controlled and medium controlled type II diabetes mellitus could cause a reduction in serum fructosamine and does not cause formation of betahydroxybutriate. [7]

MATERIAL AND METHODS

The statistical population consisted of 120 healthy medical students residing in dormitory of Tehran University. Because of some limitations, only 28 men were studied. The mean age of the students was 26.2 years. Blood samples were examined using quantitation chemiluminesence and circulating immune techniques. The methods were standardized at the Immunology department of Tehran University of Medicine.

Blood samples, without anticoagulant, were used for quantitation of CIC. To achieve this, clotted blood was centrifuged and serum was separated for CIC measurement.

Serum immune complex levels were measured and compared before and after Ramadan. The techniques employed for immune complex detection depend on a few general principles in the region of antigen – antibody equivalence of a precipitation curve, of course, the antigen – antibody complex precipitate visibly. This precipitation or aggregate formation can be enhanced by cold (used of cryoglobuline) or by the use of polyethylene glycol (PEG).

PEG is thought to enhance the activity of antigen and antibody reactants by the principle of solvent exclusion in which the PEG polymer allows water within its polymer space but excludes higher molecular weight antigens and antibodies.[8] Thus the introduction of immune reactants is enhanced promoting precipitate formation. This precipitate can be detected as in the formation of cryoglobulins. Alternatively the precipitate can be quantified by radial immunodiffusion, as described for the PEG assay.[9]

PEG working solution was prepared by dissolving 4.16 gram of PEG (6000mw) in 100ml borate buffer working solution (1000 ml). Borate buffer solution contains: Buric Acid (6.8 gram/dl), Borax powder (9.4 gram/lit), Sodium chloride (4.38 gram/lit). pH of the solution was adjusted to 8.3.

To prepare gamma globulin aggregates, 0.5ml of human gamma globulin (165 mg/ml) was mixed with 0.5 ml working borate buffer solution, shaked and incubated for 30 minutes in 63ºC and was transferred to a container, containing a mixture of ice pieces and water and kept in it for 15 minutes and after eight serial double dilutions were prepared (from 1/5 up to 1/640) reaching to the final concentration of 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 mg/ml (Table 1).

Optical density of dilutions was measured by a 20 digital spectrophotometer and a standard graph was depicted described for PEG method. [10]

To plot the standard graph, the optic density of each dilution was measured by a spectrophotometer (OD=450nm) (table 1).

The standard and blank solutions incubated in dark at laboratory temperature for 60 minutes and light absorbance of solutions was measured at 450nm wave length as shown in Table 1.

The standard graph was plotted against optical densities and standard concentration.

For each dilution, the OD of the blank was subtracted from each standard tubes OD, and the subtracted graph was plotted (Table 1).

Table 1. Results of spectrophotometric measurement. Plotting a standard graph

Tubes ilution OD 450 nm	16 mg hIgG	8 mg hIgG	4mg hIgG	2mg IgG	1mg hIgG	0.5mg hIgG	0.25mg hIgG	0.125 hIgG
Standard	436	428	416	349	337	400	245	386
Blank	300	391	300	386	185	364	184	370
Net OD	136	37	116	37	152	36	61	16

Table 2. Method of CIC assay of subjects

Test tubes Reagents	Test1 Serum1	Blank1	Test2 Serum2	Blank2
Diluted serum1	220	220	-	-
Diluted serum2	-	-	220	220
Working PEG solution	2ml	-	2ml	-
Borate buffer	-	2ml	-	2ml

The standard graph was used to measure serum CIC levels of the subject as shown in Table 2.

The results were analyzed statistically, using t test and calculated coefficient correlation between CIC before and after Ramadan.

RESULTS

In this study the serum levels of CIC of 28 healthy adult students were compared before and after Ramadan by PEG method.

The mean CIC level was 2.04 ± 1.86 before Ramadan and 2.63 ± 2.1 after Ramadan ($p = 0.05$). In all, CIC level was increased in 17 cases (60%) and was decreased in 11 cases (40%) (Table 3). But in collection of 28 cases only 6 cases (21%) were out of normal range after Ramadan, and from 6 increased cases only 1 case (3.5%) had high CIC level (out of normal range) before and after Ramadan. Two cases (7%) had relatively high CIC level (but not out of normal range) before Ramadan that stayed high after Ramadan, and only 3 cases (10%) had low CIC level (less than normal range) before Ramadan that were abnormal after Ramadan.

Same as in all of 28 cases, decreased cases (14%) which had high CIC level (out of normal range) before Ramadan , 3 cases decreased and set to normal group and one case remained in the abnormal group (Table 3)

As shown in Table 4, of 11 decreased cases (40%), 10 cases (35%) of abnormal samples set to normal group and 1 case (5%) in spite of decreasing, left in abnormal range after Ramadan.

Summing up these findings, in 25 cases (90%) CIC had increased or decreased within the normal range and in only 3 cases (10%) where CIC increased beyond normal range. Statistical analysis of two values using t test indicates that there was no significant difference in CIC levels before and after Ramadan (Table 4).

DISCUSSION

The purpose of this study was to assess the effect of fasting during Ramadan on CIC level and immune status by measuring circulating immune complex levels before and after Ramadan..

In the nephelometric PEG assay, the enhanced formation of aggregates in the presence of PEG can be quantified nephelometrically.[10,11] Precipitation of immunoglobulin by PEG is relatively nonspecific, however, and depends on the concentration of immunoglobulin present in the specimen and the concentration of PEG employed.[12, 13]

Activation of the classical pathway of the complement system is dependent on the presence of immunoglobulin G (IgG) or M (IgM) containing immune complex; thus, it is not surprising that

interaction of immunoglobulin with complement has been used to detect circulating immune complexes[14, 15].

Table 3. CIC measurement using PEG method (n = 28)

Subject	CIC level (mg/100ml) Before Ramadan	CIC level (mg/100ml) After Ramadan
1	0	1.8
2	0.09	0.18
3	0.15	7.2
4	0.36	0.9
5	0.39	6.3
6	0.45	0.75
7	0.63	2.70
8	0.75	1.80
9	0.9	2.55
10	0.9	3.60
11	0.9	4.50
12	1.50	3.90
13	1.80	2.40
14	2.10	7.20
15	2.40	6.60
16	2.70	3.90
17	4.80	5.40
18	0.30	0
19	0.45	0
20	2.10	0.45
21	2.40	0
22	2.70	1.50
23	2.70	1.80
24	3.60	0
25	4.50	0
26	5.10	1.50
27	5.70	5.10
28	6	0.18

Table 4. CIC assay

Parameter	No of subjects (n)	Before Ramadan mean ± SD	After Ramadan mean ± SD	p value
CIC	28	2.04 ± 1.86	2.63 ± 2.1	0.05

According to the Table 3, CIC level in fasting individuals which compared before and after Ramadan was not statistically significant, since in 22 cases (17%) of the healthy subjects, CIC level increased or decreased to normal range, the changes were not statistically significant. On the other hand, in 2 case (7%) of individuals tested before and after Ramadan, the CIC levels were out of normal rang, and also in this group changes of the CIC levels were not significant.

Totally the result obtained indicates that CIC levels in 22 cases (84%) of the individuals were in normal range before and after Ramadan and there were no statistically significant difference and variations (table 3).

According to the results we can find 5 cases that the level of CIC in their serum became 0 after Ramadan and this is the best condition for immunity system and this can be helpful specially in person who has auto immune disease, but unfortunately because of some limitations such as disability of ill people to become fast in Ramadan this study can not evaluate the CIC level in sick or ill people and all of the cases were healthy young men. So further researches are needed to follow the CIC level after Ramadan in patients who have autoimmune disorders or other diseases and it is better to have much more cases and also contain of women that are excluded in this study because of their menstruation period that made them stop fasting during Ramadan for a while.

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