EFFECT OF RAMADAN ON NEUTROPHIL'S RESPIRATORY BURST (INNATE IMMUNITY) AND CIRCULATING IMMUNE COMPLEX

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Backgrounds: The aim of this study was to investigate the effects of Ramadan fasting on neutrophil's respiratory burst and circulating immune complex (CIC) level. **Methods**: The effects of Ramadan fasting on neutrophil's respiratory burst and CIC was studied in 21 normal young fasting Muslim individuals using standardized chemiluminescence and poly ethylene glycol methods respectively, the results obtained and statistically analysed. **Results:** It was shown that in 11 cases out of 21 (52%) both of the chemiluminescence (CL) activity and CIC levels measured before and after Ramadan fasting were in normal range in spite of a insignificant decrease or increase in CL activity or CIC level. Therefore, the changes of the immunological parameters were not significant and the levels remained in the range of normal. In four cases out of 21 (24%), the CL activity and CIC levels were higher than normal range measured just before Ramadan, however after month of Ramadan the CL activity and CIC level decreased reaching to the normal level of these parameters. In four cases out of 21 (24%) there were an increase in CL activity and CIC levels after Ramadan fasting. **Conclusion:** There were no significant changes of CL activity of circulating neutrophils and CIC levels comparing the results obtained before and after Ramadan. More over there was a good correlation between these two immunological parameters measured in the present study.

Keywords; Neutrophil chemiluminescence Circulated Immune Complex (CIC), Ramadan

INTRODUCTION

During Ramadan the ninth month of the Islamic lunar calendar, adult Muslims are required to refrain from taking any food beverage or oral drug as well as from sexual intercourse between down to sunset. Ramadan can occur in any of the four seasons and the hours spent fasting accordingly from 11 hours to 18 hours a day. Rhythms of life is various from one country to another. In Iran two or three meals daily are eaten within a short overnight span during this month. The first meal might be taken immediately after sunset (*Iflar*) and the second one around three hours latter (dinner), the last meal might be taken shortly before down (*sahar*). Intake of drug and its adjustment to life rhythm of Ramadan is therefore not easy. Most of the people do not have any particular information about changing immunity response during Ramadan.[1] A variety of microbicidal systems are present in phagocytosis. Some are present in phagocytosis, that it also affected with circulating immune system.[2,3] The recognition of defects in the microbicidal function of phagocytes is important , since patients with such alterations are generally prone to infections.[1,2]

MATERIAL AND METHODS

The study was performed at Tehran University, Tehran, Iran, on 21 male students. They were aged 18–35 years, (mean 26.5) residing in Tehran University dormitories in 2000.

Working borate buffer solution containing Boric 6.8 gram/dL, borax powder 9.4 gram/L, Sodium chloride 4.38 gram/L, up to total volume of 1000 ml were used. The *p*H of the solution was adjusted 8.3 PEG working solution was prepared by dissolving 4.16 gram of PEG (MW 6,000) in 100 ml borate buffer working solutions. To prepare γ-globulin aggregates, 0.5 ml of human gammaglubolin (165 mg/ml) was mixed with 0.5 ml working borate buffer solution, shaked and incubated for 30 minutes in 63 ºC and was transferred to a container containing a mixture of ice pieces and water and kept it for 15 minutes and after 8 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. A standard graph was depicted described for PEG Method to plot the standard graph, the optic density of each dilution was measured by a spectrophotometer (OD=450 ηm) (Table-1). The standard and blank solutions incubated in dark at laboratory temperature for 60 minutes. Light absorbance of the solutions were measured at 450 ηm. The standard graph was plotted against optical densities and standard concentration. For each dilution, the OD of the blank was substracted from each standard tubes OD, and the substracted graph was plotted. The standard graph was used to measure serum CIC levels of the subjects.

The material used to stimulate a phagocytes' response may vary but most researchers have used either opsonised bacterial or opsonized zymosan. Chemiluminescence can also be produced in the absence of particulate matter by the soluble initiator of the metabolic burst, PMA (phorbol-myristate-acetate).

Table-1: Optical Density of Dilutions.

Reagent	Test sample	Control
Luminal 10^{-4} M	200 ml	200 ml
PMA	200 ml	–
Saline	–	200 ml
PBS	500 ml	500 ml
Normal PMN cells (2.5×10^6/ml)	200 ml	200 ml

Chemiluminescence in PMN cells (2.5×10^6/ml) stimulated by PMA

Cell preparation

About 5 ml of blood is taken by venepuncture and added to an equivalent volume of Dextran (6% w/v Dextran 110 in 0.9% w/v saline). This is allowed to settle for about 45 minutes in an upturned syringe to which a length of closefitting tubing is fitted over the needle. The lymphocyte layer was carefully removed. The plasma and the Dexteran phases were discarded. The lymphocyte slurry was then washed twice with PBS and centrifuged for 10 min at 1000 g. The cellular precipitate of lymphocytes was lysed with distilled water. This time the pellet is resuspended in 1 ml of PBS and the polymorphs counted. The polymorphs are taken diluted in PBS to a concentration of 5×10^6 cells per/ml.

Approximately 1 ml of polymorphs at this concentration can be prepared from 5 ml of whole blood and should be used with a few hours of preparation.

The chemiluminescent assay:

The LKB luminometers are easy-to-use bench top instruments capable of providing instantaneous monitoring of photometers are available from LKB–wallac for this assay. The LKB–wallac 1250 luminometer and LKB–wallac 1251 luminometer (see brochure for data reduction programs and out put devices available for this automatic lominometer. Disposable polystyrene cuvettes suitable for the luminometer using. Micropipettes with disposable tipes for dispensing 100–1000 ml.

A luminol stock solution is made by dissolving 1.77 mg of luminol in 1 ml dimethyl sulphoxide (DMSO) to give a concentration of 10^{-2} µ. Before use this was diluted further to 10^{-4} or 10^{-5} µ in PBS.

A stock solution of 2 mg phorbol-myristate-acetate (PMA) DMSO is prepared. This stock solution is diluted further by adding 50 ml to 10 ml of PBS before use.

RESULTS

The statistical population consisted of 120 healthy medical students living in University of Medical Sciences of Tehran. Because of various limitations from 120, only 21 men were studied. The mean age of the students was (25) years. They were bled before and after holly month of Ramadan. The blood samples were examined using quantitation chemileuminscence and circulating immune techniques. The methods were standardized at the Department of Immunology School of Medicine University of Medical Science of Tehran.

The blood samples were divided into two parts. The first part was extracted blood sample which was evaluated for chemileuminscence rate and the second part which was without anticoagulant, used for quantitation of CIC. In this regard, plasma was obtained after centrifugation (15 min, 1,000 g,) of another blood sample obtained as above and used for CIC measurement.

The results of chemileuminescence assay showed that the mean chemiluminescence rates before and after Ramadan were 1758.5±2054 mV and 1613±1527 mV, respectively. The means before and after Ramadan were located in normal range values (Table-2).

Statistical analysis of two values using *t*-test indicated that the differences were significant ($p<0.001$, t=0.6)

Table-2: Mean Neutrophil's Respiratory Burst and Circulating Immune Complex activity of all participants before and after holy month of Ramadan (n=21)

Parameters	1st Measurement Mean±SD	2nd Measurement Mean±SD	*p*-value***
Chemi*(mv)	1758.5±2054	1613±1527	0.0001
CIC**	2.07±4.65	2.67±5.25	0.0001

*Neutrophil's Respiratory burst measured using Chemiluminescences
**Circulating Immune Complex ***Compared by using the paired t-test.

Results of statistical analysis are shown in Table-3. In 11 cases (46%), the changes of chemileuminescence were in normal range, while 6 cases had CIC changes in normal range. In 9 cases (43%) the changes were in normal range which 2 cases had normal level of CIC, before and after Ramadan, the CIC levels in 3 cases was abnormal, however after Ramadan , the values were returned to normal, In spite, in 4 cases CL was normal, that their normal values of CIC changed to abnormal , after Ramadan.

The results of CIC assay showed that the mean CIC rates before and after Ramadan were located in normal range values (Table 2). Statistical analysis of two values using t-test indicated that the differences were not significant (P<0.001, t=0.59).

In 10 cases out of (42%), before and after Ramadan, the changes of CL and CIC values were in normal range. In 4 cases (16%), CL and CIC were abnormal before Ramadan and after that, CL and CIC values, was normal. In 3 cases (13%), CL and also CIC were increasing to abnormal range. In one case (4%) CL was increasing to abnormal range but CIC decreasing to normal range. Another one case (%4) CL was out of normal range and increasing the same normal range but CIC was normal. In one case (%4)

CL was normal and don't varied, but CIC increased and went out of normal range. And in test one case (4%), had normal CL that decreased, nevertheless, stayed in normal range, but CIC level increased and went to out of normal range.

Table-3: The result of CIC measurement using PEG method in 28 fasting Muslim subject just before and after holy month at Ramadan

Participant No.	CIC level (mg/100ml)	
	Before Ramadan	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

DISCUSSION

Every year millions of Muslims fast from dawn to dusk during the lunar month of Ramadan. A Muslim is required to abstain from any oral intake for an average time of 13 hours daily during this month.

Mean age of the subjects was 25 years (20–30). The mean HDL cholesterol level of our subjects increased significantly during Ramadan. In other studies no statistically significant change was observed in mean body weight, total cholesterol or LDL levels.[4]

Another finding shows that the eating behaviours during Ramadan may contribute to improved nutritional status of people at risk of nutritional deficiency.[3] Another study shows the Ramadan fasting in patients with well-controlled and medium-controlled type 2 diabetes mellitus could cause a reduction in serum fructosamine and does not cause formation of beta hydroxybutirate. [5]

The oxidative metabolic activity of granulocytes can be directly examined by a laboratory technique (chemiluminescence), that measures photon emission during well-defined inflammatory or microbicidal events. Numerous studies have utilized chemiluminescence to examine early changes during infectious disease and other pathologic processes. Studies have suggested that receptors on cell surfaces and oxygenation that receptors on cell surfaces and oxygenation of granulocytes can reflect the severity of disease as well as provide early diagnostic information. It is apparent that during disease myeloperoxidase and oxidase-dependent oxygenation activities reflect separate host responses and independent measurements of these activities will after a more meaningful understanding of host defence.

Immune complexes and other factors in serum may also interact with granulocytes to alter the receptors on cell surfaces and subsequent metabolic activity. In some circumstances, enhanced function of granulocytes may be determental to the host.[3] White blood cells were stimulated with complement-opsonized zymogen, c5a, F-Met- leu-phe and PMA.[1] It is well documented that autoimmune diseases are risk factors for recurrent and sever infection even when patients with these diseases are not receiving immunosuppressive chemotherapy. This predisposition may be secondary to alteration in immune function attributable to the disease process itself. Early studies suggested that granulocyte phagocytic and killing functions were most affected by these rheumatic diseases.[3]

However, subsequent investigation yielded various result for alterations in these aspects of host defences and differences have been in large part dependent on the methods employed for assessment, particularly inclusion of antilogous sera. It is now clear that immune complexes can either augment or inhibit phagocytes, and the concentration of immune complex may vary with the activity of the disease. According to measurements of luminol-enhanced chemiluminescence, normal PMNS sera of patients with active SLE stimulated the oxygenation of granulocytes and the chemiluminescence responses directly correlated with disease activity.[6]

In contrast, sera from patients with SLE in remission yielded results similar to those of control subjects. Stimulatory capacity was concentration dependent and was augmented by the addition of normal serum complement.

These observations suggest activation of PMNS by immune complexes. It is therefore likely that *in vivo* exposure of granulocytes to immune complex during relapse of disease decreases subsequent functional capacity as measured in other reports.[3]

An interesting diagnostic application of chemiluminescence is detection of neutrophil antibodies.[3]

This notice is important that accumulation phagocytes stay increased after fasting and respiratory burst and at least killing for elimination bacteria a little increased but was in normal range, Therefore innate immunity response for intracellular infection factors (gram-positive bacteria), was not decreased but was rather increasing, which is beneficial for fasting Muslims.

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