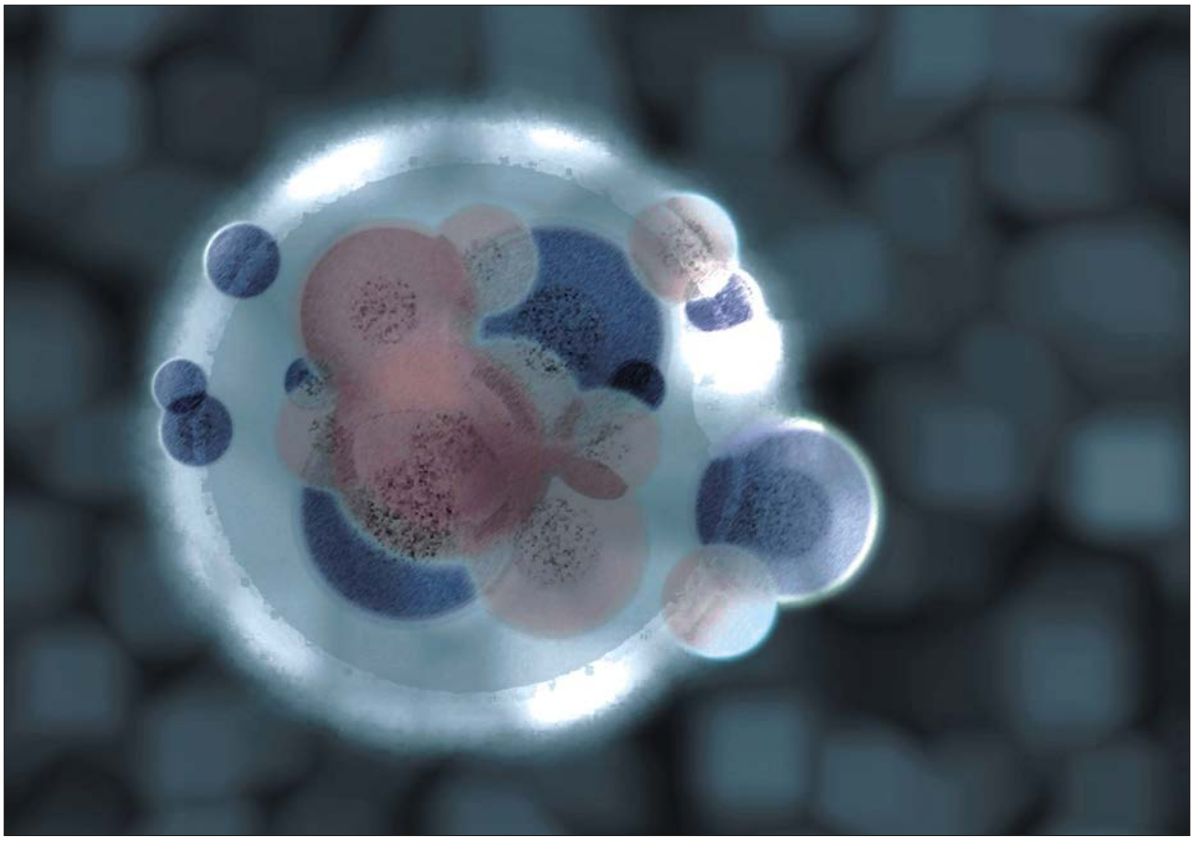

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Neutrophil Respiratory Burst (Innate Immunity) During Ramadan

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Abstract: To study the possible changes, neutrophils in 24 normal male Muslim adults were measured and compared before and after Ramadan, using chemiluminescence method. There were no significant effects of fasting on blood neutrophil function during Ramadan. Fasting during holy month of Ramadan does not change neutrophil functions and has no hazardous effect on immunity.

Key words: Neutrophil, chemiluminescence, Ramadan, immunity

INTRODUCTION

Fasting during the month of Ramadan represents one of the five pillars of the Islamic religion.

One billion Muslims around the world as soon as they reach puberty, one required to comply with this religious obligation every year. During Ramadan month, practicing Muslims abstain from eating, drinking and sexual intercourse from sunrise to sunset. People who are ill or traveling and women who are breast-feeding or menstruating are temporarily exempt from complying with these regulation. After the condition that precludes fasting resolves, individuals are required to complete a whole month of Ramadan has passed. Ramadan occurs in the ninth month of the lunar calendar, lasting between 29 and 30 days. The lunar calendar does not correspond to the Gregorian-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 (Kadri *et al*., 2000). 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 especially affected (Gharbi *et al*., 2003).

In Iran, two or three meals daily are eaten, within a short over night span during this month. The first meal might be taken immediately after sunset (*Iflar*) and the second are around 3 h later (dinner), the last meal might be taken shortly before down (sohour) (Gharbi *et al*., 2003).

The main emphasis of the authors of these studies was that most of the humans did not receive any particular information about charging their immunity response during Ramadan. Needs to be adapted according to the interaction with food intake (Gharbi *et al*., 2003). Also poly morphoneuclear (PMN) cells and monocytes play an essential role in host defense. A variety of microbicidal systems are present in phagocytosis, some dependent are present in phagocytosis, some dependent on oxygen and others are effective in oxygen absence (Easmon *et al*., 1980; Adial *et al*., 2004).

The recognition of defects in the microbicidal function of phagocytes is important, since patients with such alterations are generally prone to infections (Easmon *et al*., 1980; Gharbi *et al*., 2003).

MATERIALS AND METHODS

The study was performed at Tehran University, Tehran, Iran, Altogether 24 male students. They were aged 18-35 years (mean 26.5) residing in Tehran University.

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

Experimental method: The following experiment is presented as examples of phagocytosis and opsonisation studies involving luminal enhanced CL. These methods can be performed using either the LKB 1250 or 1251 luminometer although all results presented have been obtained using the 1250.

The principles involved in these methods can be used to design alternative assay systems, the details of which can be found in the various references cited. This

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study uses polymorphs isolated from blood and chemiluminescence is produced by the use of PMA.

Cell preparation: About 5 mL of blood is taken by venipuncture 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 min in an upturned syringe to which a length of close- fitting tubing is fitted over the needle. The upper, leukocyte rich portion (about 5 mL) is added to an equivalent volume of Phosphate Buffered Saline (PBS) and centrifuged at 500 g for 10 min after which 3 mL of distilled water is added to the pellet to hemolyze the remaining red cells. The pellet to hemolyze the remaining red cells the centrifugation step is repeated after addition of 10 mL PBS, This time the pellet is resuspended in 1 mL of PBS and then diluted in PBS to a concentration of 5×10^{-6} cells per mL. Approximately 1mL of polymorphs at this concentration can be prepared from 5 mL of whole blood and should be used within a few hours of preparation.

The chemiluminescent assay

Equipment: The LKB luminometers are easy-to- use bench top instruments capable of providing instantaneous monitoring of phagocytic events. Two luminescence photometers are available form LKB-Wallace for this assay. The LKB-Wallace 1250 luminometer and LKB-Wallace 1251 luminometer (see brochure for data reduction programs and out put devices available for this automatic luminometer). Disposable polystyrene cuvettes suitable for the luminometer being used. Micropipettes with disposable Tipes for dispensing 100-1000 mL volumes.

Reagents: -Luminol (LKB-Wallace 1243-216). 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} M. Before use this was diluted further to 10^{-4} or 10^{-5} M in PBS. PMA. A stock solution of 2 mg Phorbol-myristate-acetate (PMA) 10 mL DMSO is prepared. This stock solution is diluted further by adding 50 to 10 mL of PBS before use.

Caution: PMA may be very toxic and the suppliers recommendation for handling this product should be strictly followed phosphate- buffered saline solution (PBS). Make up in distilled water according to the following composition NaCl 0.14 M, KCl 2.7 mM, Na_2HPO_2 12 mM, KH_2PO_4 1.5 mM, $CaCl_2$ 0.9 mM and $MgCl_2$ 0.49 mM saline 0.9% w/v Nacl in distilled water.

Assay procedure: Having first prepared the polymorph suspension and pipette the following reagents in to duplicate cuvettes in the order.

Table 1: Chemiluminescence in PMN cells (2.5×10^{6} mL) stimulated by PMA

Reagent	Test sample (mL)	Control (mL)
Luminal 10^{-4} M	200	200
PMA	200	-
Saline	-	200
PBS	500	500
Normal PMN Cells 2.5×10^{6} mL^{-1}	200	200

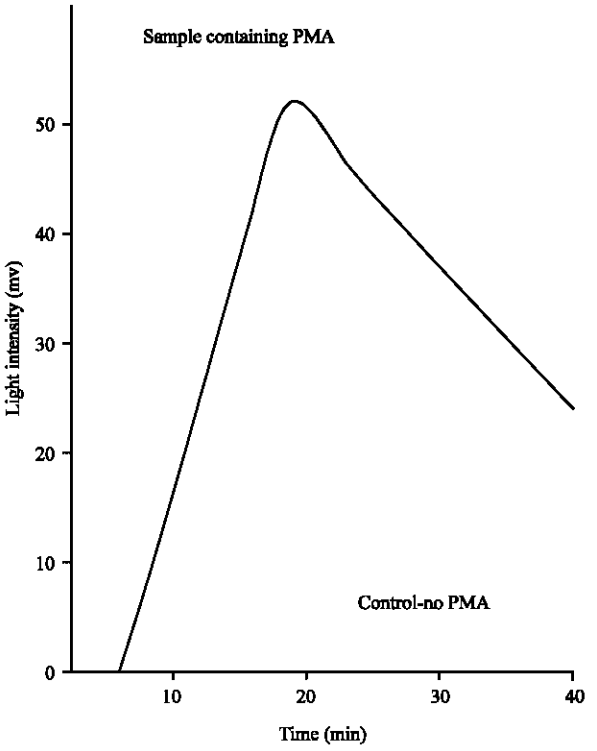

Fig. 1: The effect of PMA on chemiluminescence

Table 1 Index the cuvette-contents gently, convey the sample to the measurement position and record the light emission as mV. Measure each sample over a period of time at the selected time interval and plot the results as shown in Fig. 1. PMA stimulates phagocyte metabolic activity which results in the Fig. 1.

RESULTS

The statistical population was consisted of 120 healthy medical students living in university dormitory Because of various limitation occurred in Ramadan, including laboratory examinations blood sampling and needs for fresh blood and unsuitable blood samples, only 24 cases were evaluated. In the present study, primarily the functions of neutrophils and possible alterations of their functions were assessed during holly month of Ramadan. Neutrophils were isolated and examined using

Table 2: The result of chemiluminescence assay (millivolt/minute) in 24 fasting Muslim student

Time case number	Before holy month	After holy month
1	238	674
2	205	645
3	155	360
4	101	391
5	238	6700
6	141	6000
7	637	2236
8	939	1626
9	1469	7500
10	1655	2890
11	2757	3912
12	3570	7300
13	2420	3434
14	1243	869
15	1733	894
16	1646	273
17	2050	415
18	3700	1082
19	2850	505
20	6000	928
21	3419	743
22	240	240
23	268	262
24	1037	1022

chemileuminescence technique; All laboratory tests were standardized and conducted at the Department of Immunology, school of Medicine University of Medical Sciences of Tehran. The results indicated that the correlation coefficient before and after Ramadan, is 0.05. After Ramadan the mean of chemileuminescence was increased (Table 2). In this regard the mean value of chemileuminescence was before and after Ramadan 1620 and 2119, respectively in normal range. The two values are considered to be in normal range (Table 2).

To evaluate statistically the two mentioned values, double t-test was used. The changes were statistically significant ($p<0.0001$, $t = -0.82$). As shown in Table 2, 11 cases (45.83%) the chemileuminescence values were in normal range before and after Ramadan.

In this regard, 5 cases (20.83%) were abnormal before and after Ramadan. Therefore no significant changes were seen in the above mentioned group. In spite of minor changes in increase or decrease the chemiluminescence value and changes in this group were not statistically significant.

As shown in Table 2, 5 cases (20.83%) were abnormal before and became normal after Ramadan. Finally in third group, 3 cases (12%) which were considered to be normal, before and after Ramadan. This chemileuminescence values were found to be in abnormal value.

The results obtained from the investigation could be concluded that the fasting during Ramadan, had not injurious effects on the fasting individual, 21 Cases of 24.

DISCUSSION

Measurement of Chemiluminescence (CL) has become a well used and effective tool in studies and examinations of phagocyte functions. Chemiluminescence of phagocytes was first described by Allen who noted the emission of light quanta by granulocyte following phagocytosis. Chemiluminescence can be amplified by luminol (5 amino 2 , 3 dehydro 1,4-phthalazine dione) which is converted to an excited amino phthalate ion in the presence of oxidizing species like superoxide anion CO_2, hydrogen peroxide (H_2O_2), hydroxyl radical (OH) , and singlet oxygen (O_2). Using luminol as an amplifier, trace amounts of activated oxygen species in a sample can be measured (Andersen and Amirault, 1979). An early observation was that chemiluminescence was absent following stimulation of PMNs from patients with chronic granulomatosis disease (Gustaviani *et al*., 2004; Nelson *et al*., 1977; Steele, 1991). Thus further confirming a specific defect in the redox metabolism of leukocytes in patients with this inherited disorder. Subsequent investigations focused on chemiluminescence as an indicator of microbicidal activity (Gustaviani *et al*., 2004).

This study has shown that chemiluminescence evaluation of the activity of PMN cells from Normal subjects (Adial *et al*., 2004). Chemiluminescence is a relatively simple, inexpensive and reproducible assay for the study of bacterium to cell adherence. Phagocytosis and cellular oxidative metabolism or killing, the assay detects the generation of chemically reactive molecules resulting phagocytic cells. Light emission in this assay is directly related to the generation of products of oxygen reduction. Numerous factors are known to influence the amount of measurable light in assay. Inhibitors of chemiluminescence include bovine serum albumin, hemoglobin, arachidonic acid inhibitors of arachidonic acid, prostaglandins and cyclic AMP (Adial *et al*., 2004). Whereas agents that enhance chemiluminescence include glucose calcium/magnesium, gelatin and an alkaline extra cellular melieum. Every year, millions of Muslims fast from down until dusk during the lunar month of Ramadan. A Muslim is required to abstain from any oral intake for an average time of 13 h daily during this month. We therefore conducted a study on 24 normal subjects immune responses (adult men mean age 25 years (20-30) to observe the immunological effects of fasting. In other studies no statistically significant change was observed in mean body weight, total cholesterol level, or LDL cholesterol level. The mean HDL cholesterol level increased significantly during Ramadan (Steele, 1991). And other findings show the eating behavior during Ramadan may contribute to improved nutritional status of

people at risk of nutritional deficiency (Easmon *et al*., 1980). Another study show 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 (Ballart *et al*., 1987).

In the present primary study of neutrophil function and possible changes of their activity in 24 young male fasting students during Ramadan lated neutrophils were assayed using chemiluminescence methods, standardized of the Department of Immunology, University of Medical Science as recommended, respectively.

As appeared in Table 2, the neutrophil chemiluminescence activity of 13 cases of 24 were increased, and 8 cases were decreased and rest (n = 3) had not changes. The activity of 7 cases neutrophils were out of normal range (350 mv min^{-1}) and in the rest of the cases the activity was in normal ranges. The results indicate that 17 cases of 24 (71%) were in normal range however, in 9 cases of 17 cases mentioned above (37%) increased and 7 cases (29%) decreased and 1 case (4%) had not changes. However comparison of the results before and after Ramadan indicates mean value of chemiluminescence was before and after Ramadan 1620 and 2119, respectively in normal range (Table 2). And to evaluate statistically the two mentioned values, double t test was used. The changes were not statistically significant (p = 0.05) and other statistical analysis t-test method there is no significant changes of neutrophilchem. Activity is seen after termination of Ramadan month.

Over all the results obtained from the investigation could be concluded that, the fasting during Ramadan had not injurious affects on the most of fasting individual 21 cases of 24 (88%). With this primary study we could told fasting of Ramadan have not harmful effects in neutrophil activity that is splits of innate immunity.

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