

# Common infection and allergy induced biomarkers status in respiratory diseases among Iranian Hajj pilgrims

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## ABSTRACT

**Background:** The sources of respiratory disorders commonly occurred during the Hajj ceremony are now challenging and its infectious or allergic nature remained unknown. The present comprehensive study was conducted to assess sources of respiratory disorders in Hajj pilgrims. **Method:** In this prospective observational study, blood samples of 130 pilgrims were taken for assessment of serum levels of infectious and allergic sources. The measurement of IgA, IgG, IgM was used for assessment of infectious reactions against microbial antigens, *NBT* test was employed to assess phagocytic functions, and the measurement of gamma interferon was used for assessing immunity status against infections. Also, measurement of IgE and IL-4 was applied as two markers for assessing allergic reactions. The ELISA test was also used to assess serum levels of immunoglobulin A, G, M, E, IL-4 and gamma interferon before and three weeks after returning from Hajj ceremony. All of the volunteers were followed up along the trip and the involved pilgrim's characteristics were recorded. **Results:** The present study showed no significant change in the level of allergic biomarkers including IgE and IL-4, however the mean levels of IgM, gamma Interferon and *NBT* test were increased after the trip compared with before that. **Conclusion:** Infections play a major role in occurrence of respiratory disorders among Hajj

pilgrims and thus the role of allergic sources is doubtful.

## KEYWORDS

Hajj; Respiratory Disorder; Nitro Blue Tetrazolium Reduction Test; Gamma Interferon; Immunoglobulin

## 1. INTRODUCTION

Hajj, the journey to the sacred Mosque in Mecca, is a once in a life time obligation for all Muslims who are physically, psychologically, and financially capable. Every year, more than two million people, including approximately 100,000 Iranians, take part in the Hajj ceremony in Saudi Arabia [1]. Respiratory tract involvements are the most common problems which were occurred during this period that in about 70% of pilgrims [1,2]. However, the main causative factor/agent to produce respiratory involvements following this ceremony is unclear. In this regard, the major source of respiratory disorders as infectious agents, allergens, poisons, air dryness, or stress or even emotional factors remained unknown. The role of infectious agents including viruses, bacteria, or fungi spores in the pathogenesis of respiratory diseases among Hajj pilgrims have frequently been studied, so that many of the authors have claimed the role of infectious agents are dominant [1-5]. However, most of Iranian doctors believe that the allergens play an important role in occurrence of respiratory diseases among pilgrims and therefore, according to one report, there have been pre-

scribed different doses of corticosteroids by at least 90.3% of physicians of Hajj caravans for involved Iranian pilgrims [6].

In the present study, we employed some immunological tests for checking infectious or allergic sources for respiratory involvements among Iranian Hajj pilgrims before and after the trip. The Tests were used to determine the levels of immunoglobulin A, G, M, E, gamma interferon and NBT tests for assessing infectious sources, and levels of immunoglobulin E and interleukin-4 for assessing allergic sources. There are two types of immunoglobulin A including immunoglobulin A1 that is mainly measurable in serum and immunoglobulin A2 that is mainly available in body secretions such as tears, saliva, milk, nasal secretions and gastro intestinal secretions. This immunoglobulin has a potent anti-viral activity that its increased amounts can express viral infections. Immunoglobulin G is the major antibody that increases in secondary exposures to antigens. Immunoglobulin M is the first immunoglobulin that is produced during an immune response that increase of its level in serum indicates the acute infectious processes [7].

Gamma interferon is a cytokine produced by T cells and macrophages that involved in activation of phagocytes. It is critical cell components for innate and adaptive immunity against viral and intracellular bacterial infections [8,9]. The test of Nitro Blue Tetrazolium (NBT) is a method for assessing and monitoring of metabolic actions of the neutrophils. To destroy bacteria, oxygen radicals are necessary in the environment. NBT test focuses on the ability to produce oxygen radicals and subsequently reduce the soluble form of nitro blue tetrazolium dye to insoluble one [10]. In addition, it has been recognized that the appearance of allergy is the most common outcome of immunological reactions in human body [11]. However, in most allergic reactions, the immunoglobulin E is responsible for identification of antigens [7]. When immunoglobulin E binds to the FC receptors on mast cells and basophiles, these cells are activated and mediate releasing some chemical mediators that increase vascular permeability, vasodilatation, smooth muscle contraction, and localized inflammation in the body organs, such as the lungs and thereby can cause respiratory disease [12]. Moreover, interleukin-4 is a cytokine that plays an important role in occurrence of inflammation and allergic reactions. This cytokine produces from T helper type II cells and therefore is necessary to produce immunoglobulin E [7].

Therefore, we aimed to assess serum levels of immunoglobulin A, G, and M, as well as gamma interferon, and NBT test as infectious markers and the serum levels of immunoglobulin E and interleukin-4 as two markers for allergic disorders among Iranian Hajj pilgrims before and after the trip.

## 2. PATIENTS & METHODS

This cross-sectional study was conducted on 130 Iranian (2 paired and 260 samples) volunteers during *Hajj* ceremony. Prior to their departure, all volunteers were clinically and para-clinically examined by Caravan's physicians. The volunteers completed a consent form and at the time of entry to Saudi Arabia (*Mecca*), all those who suspected to any respiratory or allergic disorders were excluded from the study. In this study, the measurement of IgA, IgG, IgM was used for assessment of infectious reactions against microbial antigens, *NBT* test was employed to assess phagocytic functions, and the measurement of gamma interferon was used for assessing immunity status against infections. Also, measurement of IgE and IL-4 was applied as two markers for assessing allergic reactions.

We used ELISA test for assessing serum levels of immunoglobulin A, G, M, E, IL-4 and gamma interferon. The units of measurements were milligram grams in deciliter for immunoglobulin(s) and Pico grams in milliliter for gamma interferon and IL4. For NBT test, fresh blood samples were obtained and transferred to associated laboratories in Tehran University of Medical Sciences immediately and the changes were assayed by Chemiluminescence method and counting the percentage of the white blood cells which turn blue when NBT is added.

The extracted serum samples were kept at  $-20^{\circ}\text{C}$  centigrade for future analysis. All of the volunteers were followed up along the trip (one month) and the involved pilgrim's characteristics were recorded. Three weeks after the trip, the tests were repeated and the results of before and after trip were compared with together. For statistical analysis and to compare the changes in quantitative variables, paired t test or Wilcoxon test were used. Statistical significance was determined as a p value of  $\leq 0.05$ . All statistical analysis was performed using SPSS software (version 16.0, SPSS Inc., Chicago, Illinois).

## 3. RESULTS

This study is conducted on 130 volunteer Hajj pilgrims (94 male with the mean age of 47 years and 36 female with the mean age of 48.6 years). One hundred and eighty pilgrims (90.7%) were identified to suffer one or two respiratory syndromes and 12 pilgrims (9.3%) did not suffer any respiratory disease during understudied period at all. As shown in **Table 1**, no significant change was revealed in levels of allergic biomarkers including IgE and IL-4), however the mean levels of IgM, gamma Interferon and NBT test were increased after the trip compared with before that. The change in NBT reduction test was also significant after the journey. Among those no involved with a respiratory diseases during the journey (12 pilgrims), the mean level of gamma interferon in

**Table 1.** Changes in infectious and allergic biomarkers after Hajj ceremony.

Test	Before the journey n = 130		After the journey n = 130		p value
	Mean	S.D	Mean	S.D	
IgA (mg/dl)	209.4	74.0	215.2	66.7	0.490
IgM (mg/dl)	119.3	21.6	124.6	18.7	0.046
IgG (mg/dl)	1449	303.2	1451.7	236.9	0.931
IgE (mg/dl)	39.2	33.8	45.8	36.5	0.250
Interleukin4 (Pg/ml)	0.124	0.057	0.129	0.59	0.500
Gamma Interferon (Pg/ml)	2.94	2.49	4.24	4.14	0.010
NBT (%)	90.4	4.3	87.7	4.3	0.001

serum were  $3.7 \pm 5.2$  pg/ml before and  $6.4 \pm 6.0$  pg/ml after the trip, but this increased level was not significant. Also among those involved to one or more respiratory diseases during the journey (118 pilgrims), the mean serum levels of gamma interferon were  $4.9 \pm 6.0$  pg/ml before and  $5.1 \pm 5.8$  pg/ml after the trip that the difference was not meaningful, but the changes of NBT reduction test were also significant after the journey (Table 2).

#### 4. DISCUSSION

Annually, about 2 million Muslims from over 140 countries embark on Hajj [4]. Respiratory tract infections are the most common diseases that transmitted during this period [2]. The main etiologic infectious agents are not clear yet. Some authors emphasize on infectious agents, whereas, others emphasize on allergic sources for this involvement [1-5]. In this study, we assessed seven common markers for infectious and allergic disorders. As presented in Table 1, there are no significant changes in amounts of immunoglobulin A after the trip ( $p = 0.46$ ). Immunoglobulin A neutralizes the germs that enter into the mucus membranes [12]. This antibody does not activate the complement system or bind to phagocytes [13].

Immunoglobulin M increases in acute phase of the infections and is the first antibody to appear in response to initial exposure to antigens. The main function of this antibody is to stabilizing the complement to start early reply against infections. It is produced by B cells and the spleen is the major site of specific IgM production [13, 14]. In our study, IgM amounts were significantly increased after the trip ( $p = 0.046$ ). This finding can be indicated an acute infectious process among pilgrims.

Immunoglobulin G has a high production rate in the body (25 mg/kg/day) and represents the highest level in the blood and also has been increased in the chronic phase of infections. Individuals with IgG deficiency may show an increased susceptibility to infections due to encapsulated organisms [7,13]. In our study, the changes of this immunoglobulin were not significant after the journey.

When immunoglobulin E binds to the FC receptors on mast cells and basophils these cells are activated and

release of some chemical mediators. These mediators cause allergic reactions [7]. In our study, although the amounts of immunoglobulin E were increased, the changes were not significant ( $p = 0.25$ ). Therefore, allergy cannot be excluded absolutely, but it does not play the main role in respiratory symptoms in Hajj pilgrims.

IL-4 is a cytokine that has an important role in occurrence of inflammation and allergic reactions [14]. The changes of this cytokine in our study were not also significant ( $p = 0.5$ ). This finding has also been indicated that the allergic processes were not the main pathological processes.

Gamma interferon is the main macrophage activator cytokine which is important in the immune defense against intracellular pathogens. Gamma interferon is a protein made and released by lymphocytes in response to the presence of pathogens such as viruses, bacteria, or parasites or tumor cells [7-15]. In our study, the mean level of gamma interferon was significantly increased after the journey ( $p = 0.01$ ). This finding indicates an infectious source for respiratory disorders in Hajj pilgrims.

The functions of Poly Morpho Nuclear cells (PMN) are important in non-specific defense mechanisms in the immune system [7]. Most patients with acute bacterial infections demonstrate an increased reduction of NBT by their polymorphonuclear cells [14]. This test is one of the useful tests in studying neutrophils dysfunction [16]. Therefore, decreasing of the results of this test can indicate increasing risk of infections. In our study, discrepancies of NBT reduction test amounts before and after the journey were significant ( $p = 0.001$ ) and probably this finding confirms pilgrim's neutrophilic dysfunction, therefore, we cannot refuse interfering of bacterial infections. However, there have been numerous false-positive and false-negative results in this test [16].

The study had some potential limitations. We may mention, lack of any published relevant articles about immunological changes in Hajj, losing some volunteers at the return time, so that, we had to exclude 80 out of 210 volunteers from the study, lack of easy access to the volunteers and occasional cases cutting their cooperation during the journey. There was no any ethical obstacle in

**Table 2.** Amounts of serum gamma interferon and NBT percentage in involved and non-involved Iranian pilgrims to respiratory diseases before and after the journey.

Time test	Involved pilgrims N = 118				PV	Non-involved pilgrims N = 12				p value
	Before		After			Before		After		
	Mean	S.D	Mean	SD		Mean	SD	Mean	SD	
$\gamma$ -INF (Pg/ml)	4.9	6.0	5.1	5.8	0.01	3.7	5.2	6.4	6.0	0.20
NBT (%)	90.4	4.2	87.6	4.3	0.001	90.9	5.3	88.2	4.2	<0.05

this study. Results of all performed tests were also sent to the volunteers addresses.

## 5. CONCLUSION

In conclusion, the significant increase in serum level of IgM and gamma interferon after traveling, as well as the decreased function of NBT test can represent infectious processes in respiratory involved pilgrims, while no significant changes in IgE and IL-4 refuse allergic sources for this involvement. Therefore, the probability of interfering infections as the etiologic causes of respiratory syndromes among Hajj pilgrims is high and the chance of the allergies are less than the infections. Therefore, the routine administration of corticosteroids should be avoided in respiratory problems of the pilgrims.

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