INVESTIGATION ON PHAGOCYTOSIS INDEX & SERUM (IGE, IGG, IGM) IN PATIENTS OF ASTHMA IN THI-QAR PROVINCE

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ABSTRACT

The current study was conducted at the Al-Hussein Teaching Hospital in Thi-Qar province, during the period from October 2014 to May 2015. The study aimed to evaluate immune status of asthmatic patients by measuring the levels of immunoglobulin (IgE) by enzyme-linked immunosorbent assay (ELISA) and immunoglobulin (IgG, IgM) by single radial immune diffusion (SRID). The study included test phagocytic cells on phagocytosis (coefficient of phagocytosis). The study included a total of 100 patients with asthma (68 female) and (32 males) and there aged between 17-62 years. The results of the statistical analysis showed high significant increase (P ≤ 0.001) in serum IgE, IgG of patients with asthma compared with healthy control group, and no significant difference in serum IgM of patients with asthma compared with healthy control group. Decrease coefficient phagocytosis was significantly (P ≤ 0.001) in all patients with asthma compared with healthy control group.

KEYWORDS: Asthma, IgE, IgG, IgM, Phagocytosis, THI, QAR.

INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. causes recurrent episodes of wheezing, breathlessness, chest tightness, and coughing. These episodes cause airflow obstruction, often reversible either spontaneously or with treatment.[1] It is a complex disease that is caused by a combination of genetic and environmental factors.[2] These factors influence its severity and its responsiveness to treatment. Asthma is a significant public health problem, affecting approximately 300 million individuals worldwide.[3] Currently, the prevalence of allergic asthma is increasing globally.
due to air pollution and other environmental irritants. These environmental exposures are especially evident in developing countries, where industrialization is progressing rapidly.\textsuperscript{[4]}

Chronic airway inflammatory processes result in intense recruitment of activated eosinophils and T-helper (Th)2 lymphocytes at the site of injury and an inappropriate immune response to common allergens.\textsuperscript{[5]} Recurrent inflammation and subsequent abnormalities in the tissue repair mechanisms lead to structural changes in the airway wall that manifest the clinically detectable features of epithelial injury, goblet cell hyperplasia, subepithelial thickening, airway hyperplasia and angiogenesis.\textsuperscript{[6]} Thus, allergic asthma is characterized as a complex airway remodelling disease.\textsuperscript{[7]} While there is currently no cure for asthma, the standard of care for asthma is limited to symptomatic control of disease mediators with potent inhaled corticosteroids (ICS), long-acting $\beta$-adrenergic agonists and leukotriene modifiers.\textsuperscript{[8,9]}

**Immunoglobulins (Ig):** The immune system generates billions of different antibody molecules by mature B cells which are capable of secreting antibodies and expressing B cell receptors on their cell surfaces.\textsuperscript{[10,11]} Ig antibodies are large proteins composed of four polypeptide chains (two identical heavy chains and two identical light chains) joined together by disulphide bonds. Each Ig recognizes a specific antigen unique to its target and is used by the immune system to locate and destroy invading microorganisms.\textsuperscript{[12]} Serum immunoglobulin levels provide key information on the humoral immune status.\textsuperscript{[13,14]}

**Immunoglobulin E (IgE)**

is a class of antibody or immunoglobulin (isotype) that has been found only in mammals. IgE’s main function is immunity to parasites such as helminths also plays an essential role in type I hypersensitivity,\textsuperscript{[16]} which manifests various allergic diseases, such as allergic asthma, most types of sinusitis, allergic rhinitis, food allergy, and some types of chronic urticaria and atopic dermatitis. IgE also plays a pivotal role in allergic conditions, such as anaphylactic reactions to certain drugs.

**Immunoglobulin M (IgM):** It is the first isotype to be generated during a primary immune response and it is predominates in immune response to most antigens. Its pentameric structure is a highly effective activator of complement. IgM is the first immunoglobulin class to be synthesized by the neonate. IgM is larger than IgG with a molecular mass of approximately 950 kDa that makes up about 8% of the antibody in the serum.\textsuperscript{[17,18]}
Immunoglobulin G (IgG): Immunoglobulin G is a major effector molecule of the humoral immune response in man, accounts for about 80% of the total immunoglobulins in plasma of healthy individuals. The IgG (150 kD) is composed of two light chains and two heavy chains (g). The four polypeptide chains are covalently held together by disulfide bonds. It's the major antibody in the blood. Human IgG consists of four subclasses (isotypes), which are numbered in order of their serum concentrations (IgG1, IgG2, IgG3, and IgG4). IgG express predominant activity during a secondary antibody response. IgG antibodies have a relatively high affinity and persist in the circulation for a long time.[19]

Phagocytosis: The process of phagocytosis involves the internalization of large particles (≥0.5µm), phagocytosis is limited to specific Phagocytic cells such as monocytes, macrophage and neutrophils. These cells are vital to both the innate and adaptive immune systems. The innate functions of these molecules (specifically, the internalization and digestion of pathogens bound to receptor on the cell surface) represent the first line of defense against invading microorganisms. In the adaptive response, B cell produce antigen-specific antibodies lead to the opsonization of the pathogen.[20]

MATERIALS AND METHODS

study design

This study was performed on (100) Iraqi patients with bronchial asthma, who attended the Al-Hussein Teaching Hospital in Al-Nasiriya city in the period from beginning October 2014 to end May 2015. This study included too (50) person apparently healthy individuals as a control group., who have no history or clinical evidence of asthma or any other chronic disease, and no obvious abnormalities.

Blood Samples Collection

Blood samples were collected by venipuncture from 100 patients and 50 controls (five milliliters of venous blood) were drawn by disposable syringe under aseptic technique. Were placed in a sterile plane tube and allowed to clot, then serum was separated by centrifugation at 4000 rpm for 15 minutes. The serum was stored at -10 C°. These sera were used for estimating the concentration of Immunoglobulin's (Ig E, Ig G, Ig M).
METHODS

Determination of serum levels of IgG and IgM, by single radial immune diffusion (SRID) plate Principle

Principle

Kit of (Immunoglobulins, IgG, and IgM) provided by CUSABIO company. The total serum level of immunoglobulins (IgM, IgG) was determined by means of Single Radial Immune diffusion Assay. It is a single radial immune diffusion test, which was developed by Mancini et al., [21] for quantitative determination of proteins in the serum. Test sample is added to a well in an agarose gel containing a monospecific antiserum. The sample diffuses radially through the gel and the substance being assayed forms a precipitation ring with the monospecific antiserum. Ring diameter is measured and the concentration is determined from the reference standard curve.

Determination of serum levels of IgE by enzyme-linked immunosorbent assay (ELISA) Principle: This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for IgE has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IgE present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for IgE is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IgE bound in the initial step. The color development is stopped and the intensity of the color is measured spectrophotometrically at 450 nm.

Hank's Balanced salt solution (HBSS): Prepare this solution according to [22] that contains calcium ions Ca ++ and magnesium ion Mg ++ dissolving substances in 1000 ml of distilled water and adjust the pH to (PH 7.2) and divided to the volumes are equal and then sterilized By autoclave then save in temperature (4 °C) for use when we need.

Killed Yeast Suspension: was prepared for the purpose of studying the process of Phagocytosis and as the following steps
1- Dry bread Saccharomyces cervisia yeast was used.
2- 10 grams of the killed yeast was suspended in 150 ml of normal saline.
3- The suspension was placed in a boiling water bath for an hour and then it lifted until get cold then filtered via a dual-layer sterile gauze.
4- The stuck suspension was divided into many test tubes (5 mL), stored in (-20 °C) until used, and the use melted when stuck in a water bath (37 °C) and washing twice before use by using a normal, saline.[23]

**Wright’s stain**

The testing equipment (kit) was used for Wright’s stain which consists of fixative solution and Eosine Stain and solution of methylene blue which is produced by syrbio company from republic of Arabia Syria.

**Phagocytosis Procedure**

The procedure carried out according Met-Calf et al.,[24] as follows: 0.025 ml of the collected blood was put in a plane tube, then added for it 0.05 ml from Killed yeast suspension which prepared by soluble 10 grams of Saccharomyces ceversiae yeast made in Turkish pakamaya company in 150 milliliters of normal saline and put suspension in water bath for 60 minutes, then this suspension was filtered after its cooling. 0.025 ml of HBSS were added to the mixture and incubated at 37 °C for 30 minutes. One drop of the mixture was placed on a slide and smeared, then left to dry, fixed by methyle alcohol (99%) for minute and stained for 20 minutes with Wright stain. Then, examined under oil immersion.

\[
\text{Phagocytosis index} = \frac{\text{No. of phagocytic cells}}{\text{Total number of cells}} \times 100
\]

**RESULTS**

**Serum Immunoglobulins concentration**

The results of the current study show a significant difference (P ≤ 0.001) of IgE concentration (270.05 IU/ml) for patients compared with a group of control (76.85 IU/ml) with significant difference (0.001), and IgG concentration reached (1298.10 mg/dl) for patients compared with a group control (850.60 mg / dl) with a significant difference is (0.001), while IgM concentration of patients are (162.25 mg/dl) compared with the control group (139.10 mg / dl) with no significant difference (0.212).
Table (1): Shows some hematological parameters of bronchial asthma patients and healthy control

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Subject</th>
<th>No of cases</th>
<th>Mean ± SD</th>
<th>T-value</th>
<th>df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgE</td>
<td>Patients</td>
<td>40</td>
<td>270.05 ± 102.212</td>
<td>8.141</td>
<td>58</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>20</td>
<td>76.85 ± 38.470</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>Patients</td>
<td>20</td>
<td>1298.10 ± 357.145</td>
<td>3.588</td>
<td>28</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>10</td>
<td>850.60 ± 230.88</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM</td>
<td>Patients</td>
<td>20</td>
<td>162.25 ± 50.54</td>
<td>1.274</td>
<td>28</td>
<td>0.212</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>10</td>
<td>139.10 ± 37.99</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Phagocytic index

The results of the current study showed high significant difference (p ≤ 0.001) in the rate of phagocytosis, as it decreased the rate of phagocytosis asthmatic patients to (30.67) compared to the healthy controls group, and that the rate of phagocytosis (46.98) , as the table(1).

Table(2) : Shows phagocytosis index of bronchial asthma patients and healthy control

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Subject</th>
<th>No.</th>
<th>Mean ± SD</th>
<th>T-value</th>
<th>df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phagocytosis</td>
<td>Patients</td>
<td>100</td>
<td>24.81±13.26</td>
<td>5.473</td>
<td>148</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>50</td>
<td>35.80±6.31</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

In this study show, a high significant difference (p ≤ 0.001) in the concentration of immunoglobulin's (IgE) in patients group with asthma(270.05 ± 102.212) compared a healthy control group(76.85 ± 38.470),also a high significant difference (p ≤ 0.001) in the concentration of immunoglobulin's (IgG) (1289.10 ± 357.145) of the patients group with asthma, while the healthy control is (850.60 ± 230.88), and no significant difference in the concentration of immunoglobulin's (IgM) in patients group with asthma(162.25± 50.54) compared a healthy control group(139.10± 37.49).

Increased serum IgE levels in asthma may be due to increases in IgE-dependent processes and cellular components of the immune system. The secretion of IgE by lymphocytes defines the allergic state of an individual. The cellular events associated with IgE-dependent processes are very much important in asthma[25] Higher IgE levels indicate some types of inherent susceptibility and/or presence of a disease process involving airway inflammation[26] IgE is responsible for the release of various inflammatory mediators in asthma, such as histamine, prostaglandins, and leukotriens. These inflammatory mediators increase airway
narrowing due to excessive mucus production, airway smooth muscle spasm, and edema of the airway mucosa \cite{27}.

Bronchial asthma also alters serum levels of other immunoglobulins. Serum IgG levels increase along with IgE \cite{28} increased levels of IgG. Antibodies have been shown in patients with immunotherapy with offending allergen \cite{29,30} and in those with food allergy \cite{31}.

The current results show the significant difference (p ≤ 0.001) as the percentage of phagocytosis in patients with asthma (24.81 ± 13.26) compared with healthy control (35.80 ± 6.31). Our data showed that the phagocytic capacity of phagocytic cells in patient with asthma was lower than in the healthy control. A significant decrease in phagocytosis by monocytes and neutrophils \cite{32,33}, bronchial macrophages \cite{34} and alveolar macrophages \cite{35} has also been shown in asthmatic individuals in other studies.

Phagocytes in asthmatic patients had decreased capacity to move toward the yeast because that β2-agonists and corticosteroids, which are commonly used for the treatment of asthma, influence chemokine release and receptor sensitivity and, consequently, the chemotaxis of these cells \cite{36}. Corticosteroids decrease phagocytosis by monocytes and the production of inflammatory cytokines \cite{37}. Corticosteroids also increase the production of IL-10, a cytokine that deactivates monocytes \cite{38}. Monocyte chemotaxis in vitro is inhibited by high concentrations of steroids \cite{39}. In addition, corticosteroids affect nitric oxide production, total free radical production, and nitric oxide synthase activity in the monocytes of asthmatic patients \cite{40}. Therefore, it is possible that phagocytosis did not return to normal because of the action exerted by the corticosteroids on the phagocytes.

Airflow obstruction in severe asthma has been associated with eosinophilic inflammation and thickening of the airways. Eosinophilic inflammation is an important source of oxidative stress in asthma \cite{41} and oxidative stress is emerging as a common mechanism that may alter both macrophage and neutrophil functions \cite{42}.

REFERENCES


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