The complement component C9 and nitric oxide serum level in Iraqi patients with asthma

Hazima Mossa Alabassi¹, Wissam Ali Al Nadawi¹

1. Dept of Biology, college of Education for pure Science (Ibn Al-Haitham), University of Baghdad, Iraq.

*Corresponding author: Wissam Ali Al nadawi (wisamnadawi@gmail.com)

Abstract

The complement components have been associated with development of several pathophysiology feature of asthma including inflammatory cell infiltration, mucus secretion and smooth muscle cell contraction. Nitric oxide (NO) has a dual effect in asthma pathology; the production of NO by constitutive isoforms can relax the smooth muscle of airways and vessels. However, by acting in the postcapillary venule, it can induce plasma extravasation. NO can also regulate the mucosal glands, increasing the mucus secretion. This study is aimed to determine the serum level of complement component 9 (C9) and NO in asthmatic patients using ELISA technique. The results were as follows: C9 serum level significant increase (P < 0.05) in patients compared to control, while NO serum concentration has no significant difference (P ≥ 0.05) between patients and control. C9 is a component of the membrane attack complex (MAC), which forms pores in the membranes of cells of invading organisms; most nucleated cell targets resist lysis through several mechanisms, most of it induces inflammation the hallmark of asthma, elevated level of C9 has passive roles by exacerbating the inflammation, so it is a potential target for therapeutic in addition to using for diagnose the inflammation severity.

Key words: Asthma, complement components, Nitric oxide, membrane attack complex, smooth muscle, Antigen

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Introduction

Asthma is a chronic airway inflammatory disorder associated with airway hyper responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing particularly at night or early morning (Bruce et al., 2012) (Alsaffar et al., 2019). The complement is activated via three specialized pathways, the classical, alternative, and the lectin pathways, each of these pathways is activated by distinct pattern recognition receptors, that specific bind to the antigens (Harboe and Mollnes, 2008); activation of the complement cascade through the classical, alternative or lectin pathways thus generating opsonins like C3b and C5b, anaphylatoxins C3a and C5a; C3a and C5a contributing to allergic reaction by act as a pro-inflammatory mediators. Moreover, Complement
proteins are responsible for many pathophysiological features of asthma (Khan et al., 2014). The complement activation participate in airway hyperresponsiveness (Walters et al., 2002) due to their ability to recruit, activate leukocytes, trigger degranulation of mast cells, increase vascular permeability and stimulate contraction of smooth muscle (Khan et al., 2014). C5b initiates the membrane attack pathway, which results in the membrane attack complex (MAC), consisting of C5b, C6, C7, C8, and polymeric C9 (Baron, 1996). C9 is a member of the complement membrane attack complex, induces pores on the target cell membrane lead to the cell lysis (Lint et al., 1980). There are several C9 molecules in a single MAC, along with one of each of the complement components C5b, C6, C7 and C8 (Morgan, 2016). MAC is formed when C5 is cleaved to C5a and C5b, and C5b is connected in a sequential manner with C6, C7 and C8 (Tegla, 2011); multiple C9 molecules bind to C5b-8 in forming MAC. The terminal complement pathways MAC has critical role to induce inflammation in variety of inflammatory disease both in human and animals (Triantafilou et al., 2013), most nucleated cell targets resist lysis through a combination of ion pumps, membrane regulators and active recovery processes. Cells survive but not without consequence. The MAC pore causes ion fluxes and directly or indirectly impacts several important signaling pathways that in turn activate a diverse series of events in the cell, many of which are highly pro-inflammatory (Morgan, 2016). Nitric oxide is a free radical produced by a variety cells, synthesized from arginine by nitric oxide synthesis (Knowles and Moncada, 1994). Has an important antimicrobial, immunologic, and pro-inflammatory activities. (Ashutosh, 2000). In order to identify the molecular patterns associated with pathogens, innate immune system cells including, natural killer cells, neutrophils and macrophages are released as a toxic agent to kill the microbe (Tripathiet et al., 2007). In asthma, nitric oxide can have both beneficial and adverse effects. The production of NO by constitutive isoforms can relax the smooth muscle of airways and vessels via cyclic GMP regulation inducing bronchodilation and vasodilation. However, by acting in the post capillary venule, it can induce plasma extravasation; also can regulate the mucosal glands increasing the mucus secretion (Prado et al., 2011). High amounts of nitric oxide produced by inducible enzyme (iNOS) in pathological situations induce the inflammatory cell chemotaxis, particularly recruiting eosinophils and T-lymphocytes to the lung (Ricciardolo et al., 2004). The reaction of nitric oxide with anion superoxide increases the oxidative stress pathway and can induce cellular injury by protein dysfunction or DNA injury and airway hyperresponsiveness. By substrate competition, nitric oxide can control the arginase pathway and induces airway remodeling, smooth muscle contraction and mucus production (Prado et al., 2011).

Materials and Methods

Sixty individuals were enrolled in this study; the first group consisted of 30 asthmatic patients with an age range 6-18 years they attended AL-Zahra Center for Allergy and asthma in Baghdad city, for follow up. And they were steroid naïve; the second group a control group consisted of 30 subjects apparently healthy, and their age was matching to patients group. The study is carried out by ELISA technique to determine the serum level of C9 and NO by using human complement component C9 ELISA Assay kit from my bio source Company and nitric oxide enzymatic assay kit from Northwest.
Life Science Specialties Company. Three ml of peripheral blood was obtained from each individual; then the serum has been isolated using centrifuge at 3000-x for 10 min at room temperature. The assay method was carried out by follow the instructions supplied with kits.

Results

C9 serum level

Table -1- shown C9 level in studied group, the result was showed a significant elevated of C9 level in patients compared to control, (x̄ ± S.E) of patients C9 level was 108.2 ± 2.6 ng/ml while, in control 85.4 ± 2.3 ng/ml.

Table 1: Complement C9 level ng/ml in the studied groups.

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<th>Control</th>
<th>Patients</th>
<th>Probability</th>
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<tr>
<td></td>
<td>85.4 ± 2.3</td>
<td>108.2 ± 2.6</td>
<td>P &lt; 0.001</td>
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<td>Significant P &lt; 0.05</td>
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Nitric oxide serum level

Table -2- shown nitric oxide serum concentration in the studied groups. The result was showed the nitric oxide serum concentration has no significant difference between patients and control, nitric oxide concentration (x̄ ± S.E) in control was 405.5 ± 2.0 μM, and in patients 403.5 ± 2.3 μM.

Table 2: Nitric oxide concentration in the studied groups.

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<th>Control</th>
<th>Patients</th>
<th>Probability</th>
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<tbody>
<tr>
<td></td>
<td>403.5 ± 2.3</td>
<td>405.5 ± 2.0</td>
<td>P &gt; 0.05*</td>
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<tr>
<td>Significant P &lt; 0.05*</td>
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Discussion

In asthma, the complement system induces airway smooth muscles contraction; promote mucus secretion and increases blood vascular permeability. In addition to mediated key roles of airway hyper responsiveness and tissue remodeling. The present study found C9 serum level significantly elevated in patients compared to control, similar result obtained by many others, Najam et al. (2005) they were demonstrated a significantly elevated of C3 level in asthmatic patients while, C4 had a normal level. Fattahet al. (2010) have been reported a significantly higher of C3 level in Egyptian asthmatic children with a positive correlation between C3 level and asthma severity. In addition Mosca et al. (2011) has been found an increasing of C3 and/or C4 level in majority of children with atopic asthma. In contrast results obtained by Abdul-Mohymen et al., (2005), they has been found a significantly decrease of C3 and C4 level in Iraqi patients with asthma. The complement system is activated under asthma condition and the activation is related to allergens exposure. Nagata and Glovsky, (1987) have shown that extracts of several allergens including house dust mite (HDM), Aspergillus fumigatus and
perennial ryegrass allergens can induce activation complement by generation of anaphylatoxins C3a and C5a as well as IgE-mediated responses. In addition, Krug et al. (2001) also showed increasing in the levels of both C3a and C5a 24h following allergen challenge in asthmatic patients, whereas elevations in normal individuals were only minor. The most common causes of asthma exacerbations included respiratory infection bacterial and viral, allergens, irritants (Singh and Busse, 2006) (Aljubory et al., 2019), exposure to such foreign antigens represent a foreign antigens sources leading to activation the complement in different pathways (Wagner and Frank, 2010); Three major complement cascades, classical, alternative and lectin pathways, can activate the terminal pathway, including the formation of the MAC (Hadders, 2012). C9 that is component of the membrane attack complex (MAC), which forms pores in the membranes of cells of invading organisms. MAC forms when C5 is cleaved into C5a and C5b, and C5b binds sequentially C6, C7, C8 and multiple copies of the pore-forming subunit C9. Both C5a and C5b-9 regulate the downstream inflammatory cascade, which results in a massive migration of inflammatory cells into the bronchial airway lumen and triggers the release of multiple harmful inflammatory mediators (Peng et al., 2005). The membrane attack complex can induce both inflammation and apoptosis as provide by Triantafilou et al.,(2013) the activated complement mediators in asthma airway tissue remodeling are recognized as a potential targets for therapeutic intervention in preclinical and clinical research (Khan et al., 2014).

Nitric oxide

This result is consistent with the result obtained by Jang and choi (1999) where they found no different in serum level of nitric oxide in patients compared with control subjects. In contrast the result of current study is not consistent with several studies reported that nitric oxide increased in exhaled air of asthmatic subjects (Massaro et al., 1996). Warke et al (2002) founded exhaled nitric oxide was highly increased in asthmatics compared to normal children. In addition, Kharitonov et al (1994) reported that exhaled nitric oxide concentrations was high increase in asthmatic patients. Nitric oxide is important as a toxic defense molecule against infectious organisms. It also regulates the functional activity, growth and death of many immune and inflammatory cell types including macrophages, T lymphocytes, antigen-presenting cells, mast cells, neutrophils and natural killer cells (Coleman, 2001). Furthermore, Nitric oxide dilates blood vessels, raising blood supply and lowering blood pressure (van Faassen et al., 2009) causes myorelaxation, vasodilatation and anti-proliferative effects (Redington et al., 2001). Inhibition of nitric oxide synthesis leads to vasoconstriction, which raises the risk of arterial hypertension development. Current study was investigated, in a possible role of nitric oxide mediated a systemic effect in asthmatic patients. In present study, was observed no difference in the serum nitric oxide concentration in asthmatic patients and controls. Nitric oxide increases in exhaled air or sputum
but not in serum may due to that the Nitric oxide has an effect on inflammation limited to the respiratory tract in asthma as proved by Jang and Choi, (1999).

CONCLUSIONS

Increase the level of C9 in asthma will lead to the increasing of inflammation severity the hallmark of asthma, so serum level of C9 can be use to diagnose the severity ofinflammation in addition to using it as therapeutic targets.In the current study we observe a normal level of NO in asthmatic patients serum, The normal level of NO may due to has an effect on inflammation limited to the respiratory tract in asthma.

References


