The relationship between serum sialic acid and humoral immune response in patients with asthma.

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Date of acceptance 8/6/2005

Abstract

Forty-six patients with asthma were tested for the serum levels of total sialic acid (TSA). Immunoglobulins (IgA, IgE, IgG, and IgM) and leukocyte counts (total and differential). The results revealed a significant increased (P<0.0001) level of TSA in the sera of asthmatic patients (120.83 ± 6.50 vs 69.80 ± 4.36; mg/dl) and such increase was associated with significant increases in the levels of IgA (248.73±56.45 vs 64.70±14.30;mg/dl), IgE (1483.16±484.97 vs 30.33±8.21;IU/ml), IgG (1273.73±139.37 vs 572.60±51.31;mg/dl) and IgM (187.38±22.60 vs38.18±5.21;mg/dl). The data also showed non significant elevation in neutrophils, lymphocytes and monocytes. However, eosinophils were an exception and a significant increase (P < 0.0001) was observed in the patients. In conclusion, TSA could be a risk factor and a believe may be born that a modification of sialic acid residues might interfere with cell–cell recognition and interactions, which play a crucial role during immune response.

Introduction

Asthma is the most common chronic disease in developed countries. Allergy is known to play a significant role in patients with asthma. The prevalence of allergic diseases including asthma has increased significantly over the past 40 years (1). The reasons for this increase are not well understood but are under active investigation. Understanding the pathogenesis of asthma may lead to the development of novel therapies or even to preventive strategies. Little is known about the cellular and molecular mechanisms underlying this disorder.

T cells are critical for the initiation and maintenance of the mature asthmatic inflammatory response. Complex interactions between T and B lymphocytes and antigen presenting cells (APC) lead to inflammation, cytokine production, IgE production, and bronchial hyperresponsiveness (BHR) (3, 4). The ability of the immune system to provide effective defense is reflected by the great diversity of immune molecules that recognize the pathogens and by the variety of effector mechanisms that are at the disposal of the host. The concept has gradually emerged that the carbohydrate moieties of glycoconjugates act as recognition signals in the immune system (5). Sialic acids (SAs) are terminal components of many glycoproteins and glycolipids especially of higher
animals. In this exposed position they contribute significantly to the structural properties of these molecules, both in solution and on cell surfaces. Therefore, it is not surprising that SAs are important regulators of cellular and molecular interactions, in which they play a dual role. They can either mask recognition sites or serve as recognition determinant (6). When disease is present, subtle changes occur in glycosylation in malignant diseases (7), and non-malignant diseases (8). Thus, the changes in glycoprotein levels could provide clinically useful information. The objective of this study was to investigate the role of sialic acid (SA) as a possible biological marker in asthma disease and could be useful for monitoring the humoral immune response of allergic patients.

Materials and methods

Patients: This study consisted of patients treated for asthma (who were referred to consultative center for allergy and asthma, Baghdad) and healthy controls. The control group (n=21, median age 32 years, range 21-54) had no evidence of any type of allergic disease. The asthmatic patients (n=46, median age 30 years, range 10-50).

Estimation of TSA: Blood samples were collected from patients and control, and sera were separated according to Garvey et al. (9). Concentrations of TSA were estimated using the colorimetric (Resorcino reagent) method with absorbency read under optical density of 580nm (10).

Detection of Immunoglobulin IgE: Total serum IgE (IU/ml) was measured using a sandwich ELISA microplates (Biomaghreb, Tunisia). Briefly, a first mouse monoclonal antibody was immobilized to the plastic wells; a second Goat polyclonal antibody is labelled with alkaline phosphatase enzyme. The diluted samples were incubated with the solid-phase antibody-coated well. After the incubation period, the plate was washed. In a second step, the conjugate-labelled antibody was added. At the end of the second incubation, the wells were washed and third incubation was performed with the chromogen (pNPP). The reaction was stopped with NaOH and samples were read at 405nm. The level of patients IgE was determined by comparing the optical density with data established using known IgE standards in the same assay system.

Detection of Immunoglobulins IgA, IgG and IgM: Concentrations of IgG, IgM and IgA (mg/dL) were estimated using the Single Radial Immunodiffusion method. To find the value of each immunoglobulin level according to the diameter of the precipitation ring; we have used the table accompanying the test kit provided by Biomaghreb, Tunisia.

Haematological study: Total leucocyte counts were performed by using a haemocytometer. Cells were stained by the Giemsa method. A minimum of 200 cells was counted per smear to obtain a differential cell count. Cell were classified as neutrophils, lymphocytes, monocytes and eosinophils.

Statistical data analysis: Data were statistically analyzed using SPSS statistical software. Level of significant was assessed by computing independent -samples T test .Values are given as mean ± standard error. "P" values < 0.05 were considered statistically significant.

Results

1. TSA levels in asthmatic patients.
Total sialic acid was detected in healthy controls and asthmatic patients. The normal values for TSA in healthy controls was 69.80 ± 4.36 mg/dL, while in asthmatic patients was
100.83 ± 6.50 mg/dL. A significant increase (p < 0.0001) was observed in the serum levels of TSA in asthmatic patients as compared to the healthy controls (Table 1).

2. Immunoglobulin levels in asthmatic patients.

Serum level of IgA (248.73±56.45 mg/dL), IgE (148.3.16±484.97 IU/mL), IgG (1273.73±139.37 mg/dL) and IgM (187.38±22.60 mg/dL) showed significant increases (P<0.05) when compared to their respective control values (64.70±14.30 mg/dL, 30.33±8.21 IU/mL, 572.60±51.31 mg/dL, and 38.18±5.21 mg/dL; respectively) (Table 1).

3. Total leukocytes count.

The total leukocyte count in asthmatic patients was estimated to be (8450.00±516.88) cells/mm.blood showing no significant differences (P > 0.05) when compared to the normal value (6975.00±352.66) cells/mm.blood (Table 1).


The comparison between asthmatic patients and healthy controls resulted in no significant difference (P > 0.05) in neutrophils 4836.33±417.68 cells/mm.blood, lymphocytes 2681.50±149.00 cells/mm.blood and monocytes 306.16±61.74 cells/mm.blood when compared with normal value (4222.60±290.44, 2350.60±127.27 and 218.00±18.27) cells/mm.blood respectively. (Table 1). In the other hand, the value of eosinophils showed highly significant increase (P < 0.0001) (642.66±68.60 cells/mm.blood as compared to this value in the healthy controls (185.00±18.70) cells/mm.blood (Table 1).

Table 1. Total sialic acid, immunoglobulins IgE, IgA, IgG, IgM, total leukocytes count and differential leukocytes count in asthmatic patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>90% CI</th>
<th>Q1</th>
<th>Q3</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sialic acid (mg/dL)</td>
<td>150.81 ± 6.50</td>
<td>150.00</td>
<td>145.60</td>
<td>156.00</td>
<td>161.00</td>
<td>0.0001</td>
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<tr>
<td>Immunoglobulin E (IU/mL)</td>
<td>143.15±48.97</td>
<td>140.00</td>
<td>125.00</td>
<td>155.00</td>
<td>160.00</td>
<td>0.0001</td>
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<td>Immunoglobulin A (mg/dL)</td>
<td>248.73±126.85</td>
<td>250.00</td>
<td>215.00</td>
<td>300.00</td>
<td>350.00</td>
<td>0.0001</td>
</tr>
<tr>
<td>Immunoglobulin B (mg/dL)</td>
<td>1273.73±139.37</td>
<td>1300.00</td>
<td>1200.00</td>
<td>1400.00</td>
<td>1600.00</td>
<td>0.0001</td>
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<tr>
<td>Total leukocytes count (cells/mm.blood)</td>
<td>8450.00±516.88</td>
<td>8300.00</td>
<td>8000.00</td>
<td>8600.00</td>
<td>8900.00</td>
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<tr>
<td>Neutrophils (cells/mm.blood)</td>
<td>4836.33±417.68</td>
<td>4800.00</td>
<td>4500.00</td>
<td>5100.00</td>
<td>5400.00</td>
<td>0.0001</td>
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<tr>
<td>Lymphocytes (cells/mm.blood)</td>
<td>2681.50±149.00</td>
<td>2600.00</td>
<td>2400.00</td>
<td>2800.00</td>
<td>3000.00</td>
<td>0.0001</td>
</tr>
<tr>
<td>Monocytes (cells/mm.blood)</td>
<td>306.16±61.74</td>
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<td>250.00</td>
<td>350.00</td>
<td>400.00</td>
<td>0.0001</td>
</tr>
<tr>
<td>Eosinophils (cells/mm.blood)</td>
<td>642.66±68.60</td>
<td>600.00</td>
<td>500.00</td>
<td>700.00</td>
<td>800.00</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

N.S: Not significant (P > 0.05)

Discussion

Bronchial asthma is essentially a chronic respiratory disease that manifests itself intermittently as attacks of dyspnea (shortness of breath) and wheezing caused by bronchial spasms. Nearly all characteristics of the disease such as its severity, course, aggravating factors and frequency and duration of attacks, vary widely among patients. Most patients have a familial predisposition to atopic disease. Several types of asthma are recognized based on apparent etiologic factors. These include exercise asthma, cold air asthma, and industrial (chemically induced) asthma (4). While the basic mechanism of the disease is still poorly understood, all asthma attacks seem to involve a predisposing airway
hyperreactivity and the release of a battery of inflammatory mediators that cause bronchoconstriction and mucus hypersecretion (4,11). Approximately 80% of this mucus is N-glycosylated glycoproteins (12). Interestingly, this study showed there is a significant difference in the levels of serum sialic acid in patients with asthma as compared to the healthy individuals. Increased concentration of sialic acid in various tissues and fluids has been observed which may be due at least in part to defective de novo synthesis, transport, storage, catabolism, excretion and or metabolic regulation of sialic acid in the cells (13) or may be increased through changes in the bio synthesis and post translational glycosylation processing of acuteprotein phase glycoprotein in the liver (14). Allergens are identified as a key cause of allergic asthma. But the real culprit in causing allergic asthma is the IgE antibody (15). Current data also showed increases in the levels of the immunoglobulins IgA, IgG, IgM and IgE in the sera of asthmatic patients. However, since antibodies are glycoproteins in nature (16), the elevation in their levels can be directly explained by the fact that the levels of serum glycoproteins are elevated in asthmatic patients. However, our finding of the association between immunoglobulins levels and serum sialic acid concentration has not been reported before in asthmatic patients, but has been reported in patients with malignancy (7). Serum sialic acid concentration might prove to be a sensitive but not necessarily a specific marker of diagnosis of asthma, since raised levels of serum sialic acid have also been reported in several diseases (8). The IgE antibody is produced by the body in response to allergen exposure. The combination of the IgE antibody with allergens results in the release of potent chemicals called mediators. The mediators cause the inflammation and swelling of the airways, resulting in the symptoms of asthma. This makes the antibody IgE: the root cause of allergic asthma (17).

Clinical and radiological findings show that in some groups of asthmatics, the remodeling of the airways leads to permanent bronchial obstruction. There is evidence that these changes are driven by cellular mediator release in a situation of chronic airway inflammation. Eosinophils, mast cells, lymphocytes, and to a lesser extent macrophages are in increased number in allergic as well as non allergic asthma. They were shown to secrete inflammatory and non inflammatory products that play a role in inflammation and healing (18, 19). The current investigation also revealed that eosinophils have significant positive correlation with asthmatic patients which is accordance with previous reports (20, 21). Eosinophils, through release of preformed and newly generated mediators, are considered key effector cells in several diseases. Their recruitment and activation are regarded as central to the pathophysiology of allergic disorders, including asthma (22, 23, 24). And there are some explanations for the possible mechanism of increased eosinophils in patients. One of these might be the interleukin -4. IL-4 is critical to the development of allergic inflammation. It is associated with induction of the e-isotype switch and secretion of IgE: by B lymphocytes (22). IgE-mediated immune responses are probably further enhanced by IL-4 through its ability to induce upregulation of IgE: receptors on the cell surface—the low affinity IgE receptors, FceRII or CD23 (23,24) and the high affinity IgE receptor, FceRI (25). IL-4 also induces VCAM1(26,27), which, through interaction
with the α4 integrins (α4β1 [VLA-4] and α4β7) and αd β2, is able to direct the migration of T-lymphocytes, monocytes, basophils, and eosinophils, but not neutrophils, to inflammatory loci. In recent years a number of CC chemokines have been identified that cause eosinophil chemotaxis. They include rantes, eotaxin (28), monocyte chemotactic protein-3 (MCP-3) (29), MCP-4 (30), and macrophage inflammatory protein-1α (MIP-1α) (31). Rantes induces directed migration of CD4, CD45 RO+ T cells and monocytes (32), chemotaxis and activation of eosinophils (33,34), and transendothelial migration of eosinophils in vitro (35). Rantes also activates basophils and induces histamine release (36). Further, rantes and MIP-1α seem to stimulate IgE+ tonsilar B cells for IgE production (37). The T-lymphocyte also plays a pivotal role both in initiating and in sustaining immunologically driven inflammation of chronic asthma. Lymphocytes expressing the CD4 receptor are known to be important in asthma pathogenesis, through their production of particular cytokines such as IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor, which enhances eosinophil survival, maturation, and activation (38, 39, 40). The relationship between Th-2-like T-lymphocytes and eosinophils is thought to result in eosinophil accumulation in tissue independent of IgE, whereas chemokines, particularly rantes, MIP-1α, and eotaxin, are considered important for local eosinophil chemotaxis (41).

CD8+ T cells may lead to IgE class switching via IL-13 rather than IL-4. These events lead to eosinophilic bronchitis, mucus hypersecretion, and bronchial smooth muscle contraction (42). However, some studies suggest that T helper type 1 (Th1) cytokines such as IL-2, interferon gamma (IFN-γ), tumour necrosis factor alpha (TNF-α), and IL-15 may promote allergic airway inflammation as well (42,43 ). Thus, asthma as a paradigm of an exclusively Th2 mediated disease may not take into account all the complexities involved in its pathobiology. Sialic acid plays critical roles in these substances because they are soluble glycoproteins (5). These glycoproteins are referred to as acute phase reaction, thus the serum sialic acid levels may reflect the inflammatory response to the asthma. In addition, the immune functions that are affected by the changes in the sialic acid content on cell surface of immune and host cells include self/non-self discrimination, production of natural antibodies to desialylated host cells, complement activation, macrophage-mediated phagocytosis lymphocyte-mediated cytotoxicity , natural killer cells , immune cell adhesion , antigen-specific interactions , and several other important immune functions (5,6,8). We conclude that determination of sialic acid in the diagnostic evaluation of asthma needs more extensive study. We also believe that studies to clarify the exact role of sialic acid levels in the setting of inflammatory diseases will be useful as well.

Acknowledgments: we are grateful to Dr. Ali Hussain Adhiaah from tropical diseases research unit, university of Baghdad for help and support.

References


العلاقة بين مستوى حمض السياليك المتصلي والاستجابة المناعية الخلطية في مرضى الربو

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تم اختبار 44 مريضاً بداء الربو بالنسبة لمستويات حمض السياليك الكلسي في مصل الدم وعداد كريات الدم البيض (الكلي والفرقي). أظهرت النتائج حصول زيادة معنوية (P < 0.0001) في مستوى IgM و IgA و IgG و IgE والكولوبوليات المناعية (IgA). وتعدد كريات الدم البيض عند مقارنتها بالسبعة مجموعة السيطرة حيث كانت (120.83 ± 6.50 و 69.80 ± 4.36) ملغم/لسي بيتر على التوالي وافقها زيادة معنوية في مستويات الكولوبوليات المناعية عند مقارنتها بالمجموعة السيطرة عند بلغت 0.0001 ملغم/لسي بيتر على التوالي وسجلت (14.30 ± 56.45 و 64.70 ± 248.73) ملغم/لسي بيتر على التوالي وسجلت (484.97 ± 1483.16 و 8.21 ± 30.33) ملغم/لسي بيتر على التوالي وسجلت (22.60 ± 187.38 و 51.31 ± 1273.73) ملغم/لسي بيتر على التوالي. أظهرت النتائج أيضاً حصول زيادة غير معنوية في تعداد كريات الدم البيض العدلة واللقاحية وواحدة لا أنها كانت غير معنوية بالنسبة للخلايا الخلوية حيث كانت الزريدة معنوية (P < 0.0001) عند المرضى. نستطيع من هذه الدراسة أن مستوى حمض السياليك الكلسي ربما يكون عامل مهم للمرض وتعتبر كوس تغييرات في تعدد حمض السياليك ربما اثرت في التمايز والتفاعل الخلوي والتي تلعب دوراً هاماً خلال الاستجابة المناعية.