Association of Haptoglobin Phenotypes with Serum Levels of IgE and IgA in Allergic Rhinitis Patients

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ABSTRACT

Background: Allergic rhinitis (AR) is an allergic disorder of the nasal tissue that underlies diseases such as sinusitis, otits and asthma. Different predisposing factors including immunological and non-immunological factors contribute to the disease pathogenesis. **Objective:** To investigate association of haptoglobin (Hp) phenotypes (Hp1-1, 2-1 and 2-2) with serum immunoglobulins A and E levels in patients suffering from AR in comparison with healthy individuals. **Methods:** Two hundred and forty patients and 240 healthy individual entered in this case-control study. Serum levels of IgE and IgA were measured and haptoglobulin phenotypes were determined by electrophoresis. The results were evaluated by $\chi 2$ statistical test using SPSS software. **Results:** Serum electrophoresis showed that the distribution of haptoglobin phenotypes of Hp1-1, Hp2-1 and Hp2-2 among 240 patients were 11.3%, 37.9% and 50.8%, respectively. The distribution of different haptoglobin phenotypes in healthy controls were 88.7%, 36.6% and 54.7%, respectively. However, the difference between patients and controls was not statistically significant ($p=0.136$). The mean of IgE level was significantly higher in patients than controls in association with all three phenotypes ($p<0.001$). Mean of IgA serum level was also significantly different between case and control groups for Hp1-1 ($p<0.048$) and Hp2-2 phenotypes ($p<0.027$). **Conclusion:** We conclude that there is an association of all three haptoglobin phenotypes with IgE level. Hp1-1 and Hp2-2 phenotypes showed association with IgA in allergic rhinitis, as well. However, we cannot solely attribute these associations to the pathogenesis of allergic rhinitis.

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INTRODUCTION

Haptoglobin (Hp) is a tetramer glycoprotein which was first identified in serum in 1940 (1). This protein contains two heavy α and β light chains with disulfide bonds within the chains (2). Three major phenotypes named Hp1-1, Hp2-1 and Hp2-2, have been identified so far (3,4). The biological role of Hp1-1 phenotype represents the most effective factor in binding free hemoglobin and suppressing the inflammatory responses. Hp2-1 has a more moderate role and Hp2-2 has the least (5).

Haptoglobin is synthesized by liver hepatocytes and secreted in response to IL-1, IL-6, IL-8, and tumor necrosis factor (TNF) (6) and as one of the innate immune protection markers, has different biological roles (7,8).

The frequency of distribution of haptoglobulin phenotypes varies in different population (1). The only report in Iran showed frequencies of 8.2, 40.8 and 51.3 for Hp1-1, Hp2-1 and Hp2-2 haptoglobin phenotypes, respectively (9). Association of haptoglobin phenotypes with various diseases including allergic rhinitis (RA) has been studied by many researchers (3). The disease is an allergic reaction of the nasal mucosa linked to the production of allergen-specific IgE, activation of many effector cells by Th2 response and innate immunity (10,11). Several reports have shown that the immune system is activated locally and systemically in AR patients. The disease is very common in human population and the worldwide prevalence of disease is continuously increasing (12,13). Moreover, some environmental factors are important during the disease process (14,15) depending on seasonal allergic rhinitis that usually results from exposure to outdoor irritants such as trees, grass or weed pollen and perennial allergic rhinitis that is the result of indoor irritants such as dust house mites, mold spores, animal dander such as hair and skin shed by pets and feathers. The symptoms in some people prone to allergies include runny nose, nasal burning and blockage, irritation, watery eyes and sneezing (16,17).

Our hypothesis is that the distribution frequency of haptoglobin phenotypes may be associated with immunoglobulin levels in patients especially with serum levels of IgE and IgA. This study was designed to investigate this relation to better understand immunopathogenesis of the disease in combination with haptoglobulin phenotypes expression and immunological factors.

MATERIALS AND METHODS

Subjects. This case control study was carried out on 240 patients suffering from allergic rhinitis referred to ENT ward and diagnostic center for immunology and allergic diseases of two main hospitals of Zahedan city (Iran) between 2009-2010. Furthermore 240 healthy age/sex matched individuals from normal population,entered this study. All case and control subjects filled out informed voluntary consent to participate in our study according to the protocol approved by the ethics committee of the university. Blood samples (5 ml) were collected from patients and healthy subjects and 3 ml was allowed to clot and serum was separated and stored at -80°C until tests.

Immunological Tests. After taking history and completing epidemiological data, immunology and allergy tests were done. Moreover, complete blood cell count (CBC) and red cell indices were measured for each case and control individual using a calibrated electronic counter of Sysmex company. Serum levels of total IgE were

measured by a commercial ELISA assay (Padtan Elm Co,Tehran- Iran) followed by measuring serum level of IgA using SRID and nephelometry method (Binding Site LTD, Birmingham-UK).

Detecting Haptoglobin Phenotypes. Hp phenotypes were determined by gel electrophoresis and peroxidase staining based on method described on 1999 (18). Very briefly, hemolysate and sample buffer were prepared and then serum and hemolysate were mixed for saturation of haptoglobulin with hemoglobin. The samples permitted to stand for 5 min at room temperature in order to allow the Hp-Hb complexes to form. After 5 minutes, sample buffer (Loading buffer 2X) was added to this mix and were added to gel for electrophoresis. The Hp-Hb complex wa s resolved by polyacrylamide gel electrophoresis (PAGE) using a buffer containing Tris Base and glycine. For preparation of polyacralamyd gel electrophoresis (PAGE), tetra methyle etylen diamin (TEMED) and ammonium persulphate (APS), were added. Electrophoresis was performed at a constant voltage of 250 V for 3 h. After the electrophoresis was completed, the Hp-Hb complexes were visualized by soaking the gel in staining solution. The bands corresponding to the Hp-Hb complex were visible as blue color within 15 min and were stable for over 48 h. By this way, hemoglobin bound to haptoglobin with peroxidase activity oxidized a substrate called tetra methyl benzidin (TMB). The gels were photographed using digital photo camera and Hp phenotype was determined. Results from each phenotype distribution were analyzed as a percent in every group.

To compare distribution frequency of phenotype in case and control groups, $\chi 2$ statistical test was used. Mean IgE and IgA levels were compared by Mann-Whitney and Krusall-Wallis tests between the two groups for each phenotype. All statistical analyses were performed by a statistics specialist using SPSS software (Version 18). P value of less than 0.05 was considered significant.

RESULTS

In this study, 133 patients (51.2%) and 145 (60.5%) of controls were female. 218 (91%) patients were living in urban areas and 22 (9%) in rural areas. 122 patients (50.9%) were single and 118 patients married (49.1%).

Table: 1 Haptoglobin phenotype distribution in patients and healthy control.

Group	Healthy control		Patients	
Phenotype	%	Number	%	Number
1-1	8.7	21	11.3	27
1-2	36.6	88	37.9	91
2-2	54.7	131	50.8	122
Total	100	240	100	240

Mean of serum levels of IgE and IgA in different haptoglobulin phenotypes were evaluated. Electrophoretic pattern of different phenotypes in patients and healthy individuals is presented in figure 1 and the haptoglobulin phenotypes Hp1-1, Hp2-1 and Hp2-2 of patients and controls are shown in Table 1. Results showed no significant differences in the frequency of HP phenotypes between the two groups (p=0.136).

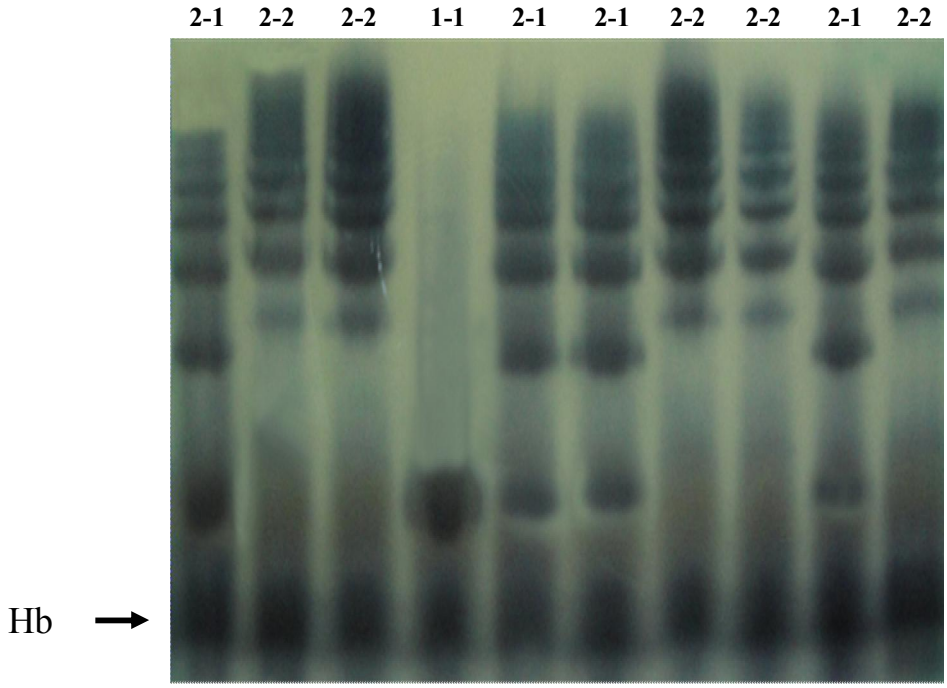

Figure 1. Electrophoretic pattern of different phenotypes in patients and healthy control.

Comparing serum IgE levels between patients and controls in each phenotypic group showed that mean of IgE serum level was significantly higher in patients than controls in all three phenotypes (p<0.001) (Table 2).

Table 2. Comparison of serum IgE levels (IU/ml) in patients and healthy control with different haptoglobin phenotype.

Phenotypes	Group	Mean	SD	P Value
1-1	patients control	341.61 76.54	± 177.43 ± 85.34	< 0.001
2-1	patients control	278.73 75.83	± 188.83 ± 98.52	< 0.001
2-2	patients control	309.56 87.90	± 162.05 ± 110.88	< 0.001

Comparison of serum IgA in sera of patients and controls with different phenotypes showed that mean of IgA serum level was significantly different between case and control groups in Hp1-1 ($p<0.048$) and Hp2-2 phenotypes ($p<0.027$). However, such difference was not observed for Hp2-1 phenotype ($p<0.073$) (Table 3).

Table 3. Comparison of serum IgA levels (mg/dl) in patients and healthy control with different haptoglobin phenotype.

Phenotypes	Group	Mean	SD	P Value
1-1	patients control	2.65 2.15	±0.91 ±1.00	0.048
2-1	patients control	2.47 2.67	±1.17 ±4.81	0.073
2-2	patients control	2.27 2.12	±1.09 ±1.17	0.027

We did not observe any difference between serum IgE and IgA levels in different haptoglobin phenotypes of AR ($p=0.083$ and $p=0.381$, respectively).

DISCUSSION

Multiple factors contribute to the etiology of allergic rhinitis. We speculated that haptoglobin might critically influence disease activity. The association of haptoglobin phenotypes with a variety of pathological conditions has interested many investigators. Therefore, Haptoglobin phenotypes have been investigated in patients with cardiac and infectious diseases (5). Association between specific protein expressions in bronchoalveolar lavage during differentiation of circulating fibroblast progenitor cells in mild asthma has been reported by Larsen K et al. (19). They described elevated levels of haptoglobin related-protein expression in patients with mild asthma compared to the other subjects and issued a novel role for expression of haptoglobin in airway remodeling in patients with asthma.

Haptoglobulin phenotype did not correlate with activation of the disease. Therefore, we assumed that other immunologic correlates should exist for the disease. We investigated the possible association of haptoglobin phenotypes expression in allergic rhinitis patients with their serum of IgE and IgA levels.

The biological activities of IgE in AR disease, has been reported by Rondon and his colleagues (10) in which, they showed increased levels of IgE had protective role in nasal mucosal secretions of individuals suffering from allergic rhinitis during their exposure to the environmental allergens.

In our study, mean serum of IgE level was significantly higher in all three phenotypes in patients compared to healthy controls ($p<0.001$).

So these three phenotypes expression maybe predictors of disease induction with combination of biological activity of IgE. Our results confirmed that haptoglobulin phenotypes expression in the allergic rhinitis patients correlates with Hp1-1 Hp2-1 and Hp2-2 expression and this might be associated with disease susceptibility in AR patients. It is, however, not clear what biological mechanism mediated the effects of haptoglobin phenotype on IgE level. This may be related to the exposure of patients to allergens via the nasal tissue or genetic determinants of serum IgE level as well as inflammatory mediators.

Furthermore, regarding the role of serum IgE in allergic rhinitis patients, it seems that haptoglobulin modulates immune response via its effect on T-cell proliferation and a dominant T-helper response as reported by Arredouani et al. (20,21). This may support the role of T helper cells and their subsets in determining the nasal inflammatory and local responses to allergens as it has been observed in a mouse model of allergic airway inflammation and airway hyperresponsivenes (21). On the other hand it can be said that the correlation of IgE level with phenotype expression, is related to the biological role of IgE in inducing humoral and cellular immune responses. Han and his colleagues indicated that increased levels of IgE and inflammatory cells, are related with Th1 and Th2 responses in patients with allergic rhinitis (22).

Regarding the IgA association with various haptoglobulin phenotypes in patients with allergic rhinitis, our results for the first time revealed that the mean of IgA serum level was significantly different between two HP phenotypes in cases and controls. These two phenotypes maybe predictors of disease induction with combination of biological activity of IgA. As the IgA plays a role in host defense against infection (23,24), it is reasonable to say that such association with different haptoglobulin phenotypes especially Hp1-1 and Hp2-2, may contribute to the mediation of allergic nasal inflammation in allergic rhinitis patients. Our result confirmed that haptoglobulin phenotypes expression in the allergic rhinitis patients correlates with Hp1-1 and Hp2-2 expression and this also might be involved in disease susceptibility in AR patients.

The nature of serum IgA activation in response to the different factors in AR remains to be elucidated.

In conclusion, the association of haptoglobin phenotypes expression with mean serum levels of IgE and IgA of allergic rhinitis patients was investigated. The results revealed that mean serum IgE levels were significantly higher in patients than controls in all three phenotypes. We also found that mean serum IgA levels were significantly different in only two phenotypes of AR patients compared to controls which may have predictive roles in the allergic rhinitis patients.

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