Platelet Endothelial Cell Adhesion Molecule-1 Polymorphism in Patients with Bronchial Asthma

Ebrahim Nadi¹, Mehrdad Hajilooii, Davood Babakhani¹, and Alireza Rafiei³

¹ Division of Pulmonary Sciences and Critical Care Medicine, Beheshti Hospital, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
² Department of Immunology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
³ Department of Immunology, Molecular and Cell Biology Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences, Mazandaran, Iran

Received: 13 April 2011; Received in revised form: 7 January 2012; Accepted: 17 March 2012

ABSTRACT

Asthma is considered as a chronic inflammatory airway disease and defined as increased tracheobronchial responsiveness to variety of stimuli. Edema and inflammatory cell infiltration in airway is observed in the asthmatic patients. One of the essential changes in inflammation is adhesion of leukocyte to endothelium and transmigration of leukocytes to the sites of inflammation. Unfortunately, little is known about the role of Platelet endothelial cell adhesion molecule-1 (PECAM-1) polymorphism in asthma inflammatory process. The purpose of this study was to determine whether PECAM-1 polymorphisms affect the risk of asthma or not.

Forty-five asthmatic patients (including 27 men and 18 women) and 45 healthy volunteers (11 men and 34 women) were studied. To determine the severity of the asthmatic situation, a questionnaire was prepared asking the following information: age, sex, clinical signs and symptoms and past medical history. All subjects were genotyped for PECAM-1 polymorphism by using amplification refractory mutation system -polymerase chain reaction (ARMS-PCR).

The genotype distribution of PECAM-1 80 Val/Met polymorphism in all asthmatic patients were Val/Val while non asthmatic controls were 95.6% Val/Val and 4.4% Val/Met. However, these differences were not statistically significant (p<0.05). The allele and genotype frequencies of PECAM-1 125 Val/Leu polymorphism were significantly different between asthmatic patients and controls. On the other hand, the presence of 125 Leu allele was associated with an increasing risk of asthma with an odds ratio of 2.8 (95% CI; 1.5-5.3, p=0.002).

Our findings suggest that the PECAM-1 125 Val/Leu polymorphism might be a genetic factor that may be associated with asthma.

Keywords: Asthma; Cell Adhesion; Genetic Polymorphism

INTRODUCTION

Asthma is a syndrome characterized by airflow obstruction.¹ Asthmatics host a special type of...
PECAM-1 Polymorphism in Bronchial Asthma

Inflammation in the airway mucosa that makes them more responsive to a wide range of triggers compared to nonasthmatics, leading to excessive narrowing with the consequent reduced airflow, symptomatic dyspnea and wheezing, that is usually reversible. The increasing global outbreak of asthma, with its large burden over patients and the high health care costs have led to extensive research about its mechanisms and treatment. An inflammation in the respiratory mucosa from trachea to terminal bronchioles exist in asthma and many inflammatory cells are known to be involved. This inflammatory process is associated with Th2 production. T lymphocytes play a leading role in coordinating the inflammatory response in asthma through the release of specific patterns of cytokines, resulting in the maintenance of a mast cell population and recruitment and survival of eosinophils in the airways. The immune system of asthmatics is tended to express the T_{h2} phenotype, whereas in normal airways, T_{h1} cells predominate. T_{h2} cells are associated with eosinophilic inflammation and increased IgE formation through the release of cytokines. Adhesion molecules such as members of the integrines, their ligands and selectin family facilitate the recruitment and movement of inflammatory cells from the blood to the airway walls and therefore play a great role in the pathogenesis of bronchial asthma.

Platelet endothelial cell adhesion molecule 1 (PECAM-1) is a 130-kD cell surface protein of the Ig-like superfamily, with six Ig-like domains in the extracellular domain. It is expressed on certain leukocytes, platelets and endothelial cells and interacts homophilically with itself or heterophilically with putative ligands such as αβ3, CD38 and CD177 to transduce downstream inhibitory signals via its cytoplasmic domain.

PECAM-1 or CD31 is an immunoglobulin superfamily molecule with 120 KD molecular weight, that plays a role in neutrophil recruitment at inflammatory sites. The interactions of PECAM-1 with its ligands are complex; the molecule is able to have homophilic adhesion as well as heterophilic adhesion (with non-PECAM-1 ligands).

The inflammatory process in the respiratory tract is associated with neutrophil recruitment at inflammatory sites and this leukocytic recruitment depends on the function of the adhesion molecules. Recent advancements in the pathophysiological understanding have suggested the involvement of the protein family of PECAM in the progression of bronchial asthma.

Platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31) plays an essential role in transmigration and leukocyte-endothelial cell adhesion. Single nucleotide polymorphisms of PECAM-1 encoding amino acid substitutions at positions 536 serine/asparagine (S/N), 643 arginine/glycine (R/G) and 98 leucine/valine (L/V) occur in strong genetic linkage resulting in two common haplotypes (LSR and VNG). These PECAM-1 polymorphisms are associated with graft-versus-host disease after hematopoietic stem cell transplantation and with cardiovascular disease. But whether PECAM-1 polymorphism influences bronchial asthma is unknown.

Thus, the major purpose of this study was to determine whether this PECAM-1 polymorphism influences the risk of bronchial asthma or not.

MATERIALS AND METHODS

Study Population

This case-control study was conducted in pulmonology clinics of Hamadan University of Medical Sciences from 2009 to 2010. Ethical approval was achieved from the Ethics Committee of Hamden University of Medical Sciences. Forty-five patients with asthma, before starting treatment or previously diagnosed asthmatic patients who didn’t receive any drug therapy within the past four weeks, were chosen from the out-patient clinics.

The inclusion criteria for all cases were bronchial asthma, where the diagnosis was established through demonstrating reversible airway obstruction. The participants were requested to fill in a questionnaire for identifying their demographic characteristics such as age, sex, asthma history, past medical history and details related to current asthma exacerbation, nocturnal and diurnal clinical signs and symptoms. In order to identify the severity of asthma, a trained observer assessed airway reversibility, peak flowmetry and spirometry in the asthmatic patients. At least three acceptable maneuvers meeting American College of Chest Physicians standards were required, with the minimum of two reproducible forced expiratory volume in 1 second (FEV₁) and forced vital capacity (FVC) maneuvers within 5% of best required for each test. The airway responsiveness was evaluated by a standardized protocol and the airway reversibility was evaluated by spirometry beforeand 15 minutes...
after inhalation of two puffs of a β-adrenergic agonist (albuterol) as metered dose inhaler. Equal or more than 12% increase in FEV1 (by at least 200 ml increase) was diagnostic for asthma. Peak expiratory flow (PEF) was also utilized to assess acute asthma severity and was expressed as percentage of the value based on age, sex, race and height. Changes in PEF are expressed as the relative changes in percentage of predicted value. According to National Asthma Education and Prevention program method, asthmatic patients were classified in 4 steps. The exclusion criteria were the presence of any inflammatory diseases and past history of recurrent infections, viral hepatitis, known collagen vascular diseases, autoimmune diseases, chronic obstructive lung disease (other than bronchial asthma), myocardial infarction/unstable angina and having been under any surgical procedures during the past four weeks. Patients who previously used inhaled steroid or systemic steroid within the past four weeks and those who were active smokers were excluded from the study. Forty-five healthy, non asthmatic adults, with no personal or family history of asthma and other inflammatory diseases were recruited from the same geographical area through blood donor clinics.

Ten ml venous blood from each subject was collected in tubes containing 50 mmol of EDTA per liter. The genomic DNA was isolated from anti-coagulated peripheral blood Buffy coat using Miller's salting out method. A polymerase chain reaction (PCR-ARMS) was utilized to detect the replacement responsible for PECAM polymorphism using Borozdenkova’s et al. method. Ten µl of the PCR product containing loading buffer, was separated in 3% agarose gel which contained 2 µg Ethidioum bromide and was visualized by ultra-violet trans-illumination.

Table 1. Characteristics of Patients and control subjects

<table>
<thead>
<tr>
<th>Gender</th>
<th>Case</th>
<th>Control</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>27 (60%)</td>
<td>11 (24.4%)</td>
<td>38 (42.2%)</td>
</tr>
<tr>
<td>Female</td>
<td>18 (40%)</td>
<td>34 (75.6%)</td>
<td>52 (57.8%)</td>
</tr>
</tbody>
</table>

| Total | 45 | 45 | 90 |

Statistical Analysis

Data analysis was conducted using the Statistical Package for Social Sciences (SPSS version 16) and data were analyzed by chi-square. P-values of less than 0.05 were considered significant.

Table 2. PECAM-1 genotype frequencies in position allele 80 in patients and control subjects

<table>
<thead>
<tr>
<th>PECAM-1</th>
<th>Asthmatic</th>
<th>Non-asthmatic</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Val-Val</td>
<td>45(100)</td>
<td>43(95.6)</td>
<td>88(97.8)</td>
</tr>
<tr>
<td>Val-Met</td>
<td>0(0)</td>
<td>2(4.4)</td>
<td>2(2.2)</td>
</tr>
<tr>
<td>Met-Met</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
</tbody>
</table>

Table 3. PECAM-1 genotype frequencies in position allele 125 in patients and control subjects

<table>
<thead>
<tr>
<th>PECAM-1</th>
<th>Asthmatic</th>
<th>Non-asthmatics</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Val-Val</td>
<td>12(26.7)</td>
<td>31(68.9)</td>
<td>43(47.8)</td>
</tr>
<tr>
<td>Val-Leu</td>
<td>26(57.8)</td>
<td>8(17.8)</td>
<td>34(37.8)</td>
</tr>
<tr>
<td>Leu-Leu</td>
<td>7(15.6)</td>
<td>6(13.3)</td>
<td>13(14.4)</td>
</tr>
</tbody>
</table>

RESULTS

Patient Data

The characteristics of the population are presented in Table1. Patients had a mean age of 53.9±12.6 years (min=23, max=80) and control subjects with a mean age of 51.8±14.9 years (min=24, max=85), respectively. Twenty seven (60%) of patients and 11 (24.4%) of controls were males.

As the table 2 shows the genotype distribution of PECAM-1 80 Val/Met polymorphism in all asthmatic patients were Val/Val while non asthmatic controls were 95.6% Val/Val and 4.4% Val/Met. However, these differences were not statistically significant (p<0.05).

PECAM-1 genotype frequencies in position allele 125, in 12 asthmatic patients were Valine-Valine (26.7%), in 26 patients were Valine-Leucin (57.8%) and in 7 patients were Leucin-Leucin (15.6%) but in non-asthmatic patients, in 31(68.9%) were Valine-Valine and in 8(17.8%) were Valine-Leucin and in 6(13.3%) were Leucine-Leucine, with significant differences between the patients and control subjects (chi-square =18 and p<0.01) (Table 3). On the other hand, the presence of 125 Leu allele is associated with an increasing risk of asthma with an odds ratio of 2.8 (95% CI; 1.5-5.3, p=0.002). Furthermore, since the occurrence of 125 Val/Leu polymorphism might change PECAM-1 gene function, heterozygosity in that position is accompanied by higher likelihood of development of asthma when compared to that in controls (OR=8.4, 95% CI; 2.9-23.6, p=0.00002).
PECAM-1 Polymorphism in Bronchial Asthma

Table 4. PECAM-1 allele polymorphism frequencies in asthmatic patients and control subjects

<table>
<thead>
<tr>
<th>PECAM-1</th>
<th>Asthmatic</th>
<th>Non-asthmatic</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 Val</td>
<td>90(100)</td>
<td>88(97.8)</td>
<td>178(98.9)</td>
</tr>
<tr>
<td>80 Met</td>
<td>0(0)</td>
<td>2(2.2)</td>
<td>2(1.1)</td>
</tr>
<tr>
<td>125 Val</td>
<td>50(55.6)</td>
<td>70(77.8)</td>
<td>120(66.7)</td>
</tr>
<tr>
<td>125 Leu</td>
<td>40(44.4)</td>
<td>20(22.2)</td>
<td>60(33.3)</td>
</tr>
</tbody>
</table>

PECAM-1 allele polymorphism frequencies for 80 Valine allele in asthmatic patients were 90(100%) but in non-asthmatic patients were 88(97.8%) and in 2 patients were 80 Methionine (2.2%), with no significant differences between the patients and control subjects \( (p>0.05) \).

The frequency of major 125 Val allele was 55.6% and 77.8% in asthmatic patients and controls, respectively. In addition, as it was shown in table 4 the frequency of PECAM-1 125 Leu allele was more prominent in asthmatic patients than that non asthmatic controls \( (44.4\% \, vs. \, 22.2\%, \, p =0.02) \).

**DISCUSSION**

The main finding of our study was an association between the presence of Leucine amino acid at position 125 and increased risk of asthma. Chronic inflammation which is a hallmark of pathogenesis of asthma is a distinct inflammatory process initiated by vascular changes that lead to recruitment of circulating leukocytes to inflamed vascular wall and lung tissue. Egression of circulating leukocytes from the vascular endothelium at sites of allergic inflammation is dependent upon several steps of interactions between adhesion molecules and their receptors which mediate transmigration of distinct leukocytes across endothelium. Several adhesion pathways from initial leukocyte rolling along endothelium (such as P-selectin glycoprotein ligand 1 and L-selectin) to firm adhesion (such as ICAM-1, PECAM-1 and VCAM-1) to endothelial cells, mediate leukocyte tissue recruitment. 23-24 PECAM-1 is an extracellular adhesion molecule which plays an important role in neutrophil, monocyte, and NK cell and eosinophil transendothelial migration. PECAM gene is encoded by 16 exons with 6 extracellular domains. 25 So that the PECAM-1 molecule might be as potential biomarker for asthma due to its large tissue distribution, the role in transendothelial migration, and the structure of its extracellular domain. 26 The interaction between neutrophil, monocyte or eosinophil PECAM and endothelial PECAM is a homophilic interaction which may be mediated by interdigitating PECAM molecules from leukocytes and endothelial cells forming a zipper which promotes their adhesion. 27

A polymorphism in PECAM-1 gene (Leu125Val) in Exon-3 encoding first extracellular Ig-like domain that mediates the homophilic binding of PECAM-1 has been documented. In fact, the presence of an association between asthma and the PECAM-1 heterozygous state at the Val125Leu codon \( (OR=8.4, \, 95\% \, CI; \, 2.9-23.6, \, p=<0.0001) \) emphasizes that studying the PECAM-1 polymorphism may be a new interesting way to investigate the role of the immunoregulatory markers in the asthma pathogenesis. On the other hand, we noticed that the Leu125 allele, in a genetic recessive model, may be a significant risk factor in the paradigm of allergic asthma. Val125Leu polymorphism occurred in a codon which belongs to the extracellular domain of the PECAM-1 molecule that seems to be essential for the cell-cell adhesion events and may also be involved in the signal transduction process 28. Furthermore, it should also be noted that the association of gene polymorphisms and asthma does not persuade that the identified gene polymorphism plays a direct role in the development of the disease. Therefore, the association might be explained by the fact that the functional polymorphic sites are in linkage disequilibrium with other gene triggering factors such as inflammatory cascade molecules, cytokines, and chemokines. This speculation is in line with our previous report which showed the effect of Ser128Are polymorphism in E-selectin gene on severity of asthma, in addition, other studies also demonstrated a strong association between the polymorphisms of the IL-4 gene promoter at positions -590, -33 and -1098 and bronchial asthma. 29, 30 In another study in China, over expression of the cell adhesion molecule CD44 was closely related to inflammatory cell infiltration in the airways of asthmatic patients. 31

Since Val125Leu polymorphism of PECAM-1 gene in the first Ig-like domain of PECAM involved in the homophilic interaction is a functional polymorphism, it appears that this polymorphism can act in two ways: first to generate recruitment of leukocytes on bronchial periarterial network in a susceptible subject; second as
immunoregulator by affecting the transendothelial migration of distinct leukocytes.\textsuperscript{32}

We could not find any association between another PECAM-1 polymorphism (Val80Met) and asthma, because of our small sample size which might decrease the power of study; it would be difficult to rule out the effect of this polymorphism on initiation of inflammatory process in asthma. It was then necessary to analyze a large number of patients and controls to confirm and assess the relevance of the 125Leu and 80Met alleles in asthma.

Taken to gather, our results demonstrated for the first time that the PECAM-1 polymorphisms may be novel genetic markers of susceptibility to asthma at least in the Iranian patients.

ACKNOWLEDGEMENTS

The authors thank to Dr. Farzaneh Asnaashari for her assistance in research experiment and Miss Marzieh Naderi Shahab for editing the manuscript.

REFERENCES

6. Etzioni A, Stiehm ER, Feldweg AM. Leukocyte-endothelial adhesion in the pathogenesis of inflammation. up to date 17.3
PECAM-1 Polymorphism in Bronchial Asthma

Institute of Health, Bethesda MD, 2002, Publication no.92,3659.


