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Title: Increased Levels of Plasma Soluble Human Leukocyte Antigen G in Persistent Wheezy Infants.

Running Title: Persistent Wheezy Infants and sHLA-G.

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ABBREVIATIONS

HLA: Human leukocyte antigen

MHC: Major histocompatibility complex

AR: Allergic rhinitis

mAPI: Modified asthma predictive index

Ig: Immunoglobulin

NK : Natural killer

BAL: Bronchoalveolar lavage

IL: Interleukin

IFN: Interferon

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ABSTRACT

BACKGROUND: Human leukocyte antigen (HLA)-G is a non-classical major histocompatibility complex (MHC) class I antigen characterized by limited polymorphism in its coding region, unique tissue expression pattern in physiologic conditions and immunomodulatory properties. Recently, many studies suggest that the levels of sHLA-G were higher in atopic asthma and allergic rhinitis (AR). However, this remains to be uncertain in wheezy infants. In the present study, it was aimed to determine sHLA-G levels in wheezy infants.

METHODS: The study included infants with persistent wheezing and positive modified asthma predictive index (mAPI) (n=30) (persistent group) and those with transient wheezing and negative mAPI (n=17) (transient group). sHLA-G levels were determined in plasma by using enzyme-linked immunosorbent assay. Total immunoglobulin E (IgE) and eosinophil counts were obtained whereas skin testing was performed with a battery of 13 antigens with appropriate positive and negative controls.

RESULTS: When infants with persistent wheezing and positive mAPI (persistent group) and those with transient wheezing and negative mAPI (transient group) were compared, sHLA-G levels were found to be significantly higher in persistent group ($p = 0.008$). There was not a significant difference in the peripheral blood eosinophil counts and total IgE levels among the groups.

CONCLUSIONS: To best of our knowledge, this is the first study assessing sHLA-G levels in wheezy infants. The finding of increased sHLA-G levels in infants with persistent wheezing suggests that it may be a parameter which could be used to distinguish which wheezing would be persistent. Further comprehensive studies are needed in this topic.

Keywords: Asthma, Children, sHLA-G, Persistent Wheezing

INTRODUCTION

The class I human leukocyte antigen (HLA) genes are located at the telomeric end of the HLA complex on chromosome 6p21 and include both class Ia (HLA-A, -B and -C) and class Ib genes (HLA-E, -F and -G) [1, 2]. HLA-G is unique among class I genes because it undergoes alternative splicing. Seven alternatively spliced transcripts have been identified; four of these transcripts are predicted to encode membrane-bound proteins (HLA-G1, HLA-G2, HLA-G3, HLA-G4), while three are predicted to encode soluble proteins (HLA-G5, HLA-G6, HLA-G7) [3, 5]. The expression of HLA-G was first described in the 1980s in choriocarcinoma cells and primary trophoblasts in the placenta [6]; therefore, the first studies on HLA-G evaluated its role in pregnancy. The fact that maternal immune system has a communication with tissue and cells of semi-allogeneic fetus during pregnancy suggests specific mechanisms that impede rejection of fetus by maternal immune system including fetal tolerance. HLA-G has various immunoregulatory effects on the cells of immune system including inhibition of cytotoxic T lymphocytes, natural killer (NK) lysis, induction of apoptosis in T lymphocytes and NK cells, inhibition of T cell chemotaxis and modulation of pro-angiogenic factor release from NK cells. The constitutive HLA-G expression occurs in adult thymus, cornea, pancreatic islets and endothelial and erythroid precursors; in addition, conditions such as organ transplantations, inflammatory diseases, cancers, autoimmune diseases, viral infections and multiple sclerosis can also induce HLA-G expression [7].

Asthma is a chronic lung disease characterized by persistent airway inflammation, bronchial hyperresponsiveness, and structural remodeling which is mainly directed by Th2 cytokines [8]. Lower concentrations of sHLA-G enhance the expression of Th1-type cytokines, but exposure to higher sHLA-G concentrations is associated with development of Th2-type cytokine response [9]. In a previous study using positional cloning, HLA-G was identified as an asthma susceptibility gene; and sHLA-G expression was demonstrated in airway epithelial cells [1]. Authors also demonstrated that sHLA-G levels were increased in the bronchoalveolar lavage (BAL) fluid of asthmatic adults compared to healthy controls [10]. Moreover, it was also shown that circulation plasma levels of sHLA-G was elevated in children with atopic asthma [11] and those with allergic rhinitis (AR). [11-13].

The term “wheezing infant” is used for those who have 3 or more wheezing attacks but no underlying disease in the examinations [14]. The wheezing may onset at various ages and may disappear by time in some children; however, it may have a persistent course and result in asthma. Frequent wheezing episodes within first three years of life, history of parental asthma, history of eczema, wheezing independent from AR. and flu, presence of eosinophil count $\geq 4\%$ in blood are primary risk factors for asthma [15]. In addition, aeroallergens and some foods are also considered as risk factor for allergic sensitization [16]. Yet, there is no definitive criterion that identifies which wheezing would be persistent. Levels of sHLA-G, found to be increased in asthma, are unknown in wheezing infants.

In the present study, the aim was to determine levels of sHLA-G levels in wheezy infants.

METHODS

Study Population

This prospective study was conducted in the Department of Pediatric Allergy outpatient clinic of a tertiary center. In all patients, demographic, clinical and laboratory data were recorded (Table 1). Total IgE levels were measured by using nephelometric method and eosinophil count was performed by using Siemens Acvia 2120 analyzer. Skin tests were performed by using 13 aeroallergens and 8 food allergens with positive and negative controls. Induration 3 mm greater than negative controls was considered as positive.

Modified asthma predictive index (mAPI) was considered as positive in the presence of either one major criterion (parental history of asthma, physician diagnosed atopic dermatitis, allergic sensitization to at least one aeroallergen) or two minor criteria (wheezing unrelated to colds, eosinophils above 4% in circulation, allergic sensitization to milk, egg or peanuts) in addition to recurrent wheezing episodes [16].

Tucson Children's Respiratory Study which classified infant wheezing in the first three years as either transient or persistent, depending on the absence or presence of symptoms [17]. The same procedure has been used in other studies [18,19]. Although the persistent wheezing is commonly associated family history of asthma and/or with atopy and atopic markers, allergic sensitization, significant loss of lung function in the first years of life, and airway hyper reactivity, the transient wheezing phenotype is not related with a family history of asthma and/or with atopy. In this study infants with wheezing aged 9-44 months were assigned into groups as follows: persistent group including infants with positive mAPI (n=30) and transient group including those with negative mAPI (n=17). Patients with immunodeficiency, heart and lung disease, those received corticosteroids within prior month and premature infants were excluded. All blood samples were taken into EDTA tubes and plasma were separated. All samples were stored at -80°C until assays.

Ethical Disclosures

This study was approved by the Ethics Committee of Erciyes University and was conducted in accordance with the World Medical Association and the Helsinki Declaration (<http://www.wma.net/s/ethicsunit/helsinki.htm>). All parents gave written informed consent before participation.

sHLA-G Measurements

sHLA-G levels were measured using a specific sandwich enzyme-linked immunoabsorbent assay. The ELISA kits used to detect total sHLA-G levels were obtained from BioVendor (Palackeho tr. Brno, Czech Republic). The sensitivity of kits for sHLA-G was 2 units/ml.

Statistical Analysis

Statistical analysis was performed using SPSS version 20.0. The level of sHLA-G, total IgE, and blood eosinophil counts were compared using the Mann-Whitney U test. A p-value < 0.05 was considered statistically significant.

RESULTS

Patients Characteristics

Of the 47 children included, 30 had persistent wheezing while 17 were employed as transient wheezy infants. The age range was 9-44 months. Table 1 presents characteristics of cases.

No significant differences were detected between children with persistent wheezing and controls regarding absolute eosinophil counts and percent of eosinophil ($p>0.05$; Mann Whitney U test) (Table 1).

No significant differences were detected between children with wheezing and controls regarding total immunoglobulin (Ig) E ($p>0.05$; Mann Whitney U test) (Table 1).

Plasma sHLA-G levels

When sHLA-G levels were compared between children with persistent wheezy infants and transient wheezy infants, sHLA-G levels were found to be markedly higher in persistent wheezy infants group (67.9 ± 25.2) than transient wheezy infants group (51.4 ± 25.2) ($p = 0.008$; Mann Whitney U test) (Figure 1).

DISCUSSION

As known, wheezing is generally transient in infants. Eighty percent of the infants who have a wheezing episode within first year of life will not experience wheezing episodes after 3 years of age. Similarly 60% of the infants having a wheezing episode in the second year of life and 30-40% of the infants having a wheezing episode in the third year of life will not experience wheezing episodes after 3 years of age [20]. However, wheezing could generally be persistent in infants with positive mAPI. Our data demonstrated that serum sHLA-G levels were significantly higher in persistent wheezy infants than in transient wheezy infants, indicating increased production of sHLA-G molecules in the plasma of persistent wheezy infants. It was thought that sHLA-G level was high in persistent wheezing group because of positive mAPI. Our results are consistent with a previous study which reported higher plasma sHLA-G levels in 27 school children with atopic asthma compared to 26 children with non-atopic asthma or 16 controls [11].

Th2-polarized inflammation is used to describe asthma. Th2-originated cytokines such as interleukin (IL)-4 and IL-13 in concert are the primary interleukins regulating inflammatory reaction [8]. IL-4 and IL-13, which are selective for eosinophil recruitment, induce IgE synthesis, up-regulate adhesion molecules; thus, lead a raised in mucus production and airway hyperreactivity [8]. It was suggested that Th2-type cytokine response is associated with exposure to elevated sHLA-G concentrations while low sHLA-G concentrations cause an increase in the expression of Th1-type cytokines [9]. Thus, authors concluded that HLA-G can play role as tissue protective molecule during inflammation [9]. In the study by Maejima et al., it has been suggested that stimulation of Th2 cytokine secretion by HLA-G is mediated by mononuclear cells in the peripheral blood [21]. In recent years, increased circulating levels of G5 were demonstrated in 2 studies including children with atopic asthma [11, 22]. In another study, it was found that sHLA-G was present in substantially higher amount in BAL fluid obtained from subjects with mild, persistent asthma when compared to those obtained from controls [10]. In contrast, in the *in vitro* study by Rizzo et al., it was shown that there was a specific deficiency in IL-10 secretion and a reduce in sHLA-G production in the asthmatic patients [23].

Genetic factors have a role in asthma pathogenesis. Over 100 genes have been implicated in asthma or atopy susceptibility. HLA-G may be one of these genes. In 2005, Nicolae et al. demonstrated that genetic polymorphisms (-944A/G) in HLA-G confer susceptibility to airway hyperresponsiveness and asthma; thus, HLA-G is an asthma susceptibility gene by fine-mapping and positional candidate studies [1]. A study by Tan et al. [24] indicated that a single nucleotide polymorphism (SNP) in the 3' untranslated region of HLA-G gene has a possible functional role in asthma susceptibility. In our study, sHLA-G levels were found to be increased in persistent wheezing group which had positive family history for asthma, genetic asthma susceptibility and positive mAPI.

As similar to asthma, AR is also defined as a disease characterized by mucosal and submucosal inflammation which is driven organized by Th2 cells. It was shown that cytokines including IL-4, IL-5 and IL-13 induce IgE synthesis and eosinophil accumulation in the nasal mucosa in AR [25]. Thus, a

role similar to that in asthma can be proposed for HLA-G in allergic rhinitis. Ciprandi et al. are the first authors who first demonstrated significantly increased serum sHLA-G and sHLA-A, sHLA-B, and sHLA-C levels in AR patients with pollen allergy [12]. Recently, authors extended their findings to patients with perennial AR. In the latter study, it was shown that circulating sHLA-G levels were increased when compared to control subjects and that there was a strong correlation between sHLA-G and symptom severity which was determined by the visual analogue scale and use of rescue medication, namely levocetirizine [26]. The same group evaluated use of sublingual immunotherapy (SLIT) in the patients with AR [13, 27]. They provided the first published evidence that the decrease of sHLA molecules serum levels and the increased interferon (IFN)-gamma production after SLIT in AR patients with pollen allergies [27]. In addition, HLA-G and sHLA-A, sHLA-B and sHLA-C were measured before and after pre-seasonal SLIT in 40 patients with pollen allergy. It was found that there were significant decreases in sHLA-G and sHLA-A, sHLA-B and sHLA-C levels after SLIT, which were found to be significantly correlated with improvement in symptom severity. However, circulating IL-4 and IFN-gamma levels were not affected [13]. The finding of increased sHLA-G levels in persistent wheezy infants, in whom pulmonary inflammation is considered to be more intensive compared to transient wheezing, is consistent with increased sHLA-G levels in AR, an inflammatory disease.

Absence of a control group is the limitation of our study. The present study provides preliminary evidence for significantly increased serum sHLA-G levels in persistent wheezy infants. When studies on levels are taken into account, our results are consistent to literature. The finding of higher sHLA-G levels in wheezing infants suggests that it could be a parameter that could be used to differentiate which wheezing would be persistent. Further studies are needed to confirm the preliminary findings.

Disclosure

The authors declared no conflicts of interest.

Author contribution

F.T. performed the data analysis, contributed in discussion and supervised the whole study, H.E.G. contributed in study design, data collection and wrote the article, H.H.A. collected data, B.S. measured plasma sHLA-G levels. All authors read and approved the final manuscript.

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Figure legends

Table 1. Features of persistent wheezy infants and transient wheezy infants.

* Median(Interquartile range)

† Mann Whitney U

‡ Chi-square

Figure 1. Plasma sHLA-G (soluble Human Leukocyte Antigen-G) levels in persistent wheezy infants and transient wheezy infants.

Table 1. Features of persistent wheezy infants and transient wheezy infants.

	Persistent wheezy infants (n=30)	Transient wheezy infants (n=17)	P value
Age (month) *	28.5 (9-44)	28 (17-43)	>0.05 †
Sex			
Female n (%)	10 (33.3)	5 (29.4)	>0.05 ‡
Male n (%)	20 (66.7)	12 (70.6)	
Eosinophil counts (%) *	3.5 (0.2-13)	4.8 (0.07-9.6)	>0.05 †
Eosinophil counts /mm³ *	325(20-1930)	420 (30-1350)	>0.05 †
IgE (ku/L) *	24.5 (2-1980)	34.9 (13-4320)	>0.05 †

* Median(Interquartile range)

† Mann Whitney U

‡ Chi-square

