

Evaluation of Soluble HLA-G Serum Level as Diagnostic Biomarker in Allergic Rhinitis Patients and its Association with Specific IgE Levels

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About 30 % of people are affected by allergic rhinitis (AR). Allergic rhinitis is an inflammatory condition defined by a disturbance of immunoregulation generating an amplified T helper "Th-2" response. An inflammatory mechanism mediated by HLA-G is thought to be involved through initiation of Th2 cytokine profile releasing "interleukin IL-3, IL-4, and IL-10". We evaluated levels of soluble HLA-G "sHLA-G" and studied its association with "allergen specific IgE" in AR patients. Twenty-five AR patients and twenty healthy subjects were enrolled. sHLA-G levels (U/ml) were measured by an immunoenzymatic method, while specific IgE levels (IU/ml) of respiratory allergens were analyzed using immunoblotting assay. The results revealed that AR group has significantly higher serum sHLA-G levels than normal subjects, $P<0.001$. There was a highly significant and positive correlation between sHLA-G levels and specific IgE levels of *Alternaria alternate* and *Dermatophagoides pteronyssinus* ($P<0.01$). Besides, there was a significant ($P<0.05$) positive correlation between sHLA-G levels and specific IgE levels of brich and mixed grasses. We concluded that serum sHLA-G level is significantly increased in AR patients and that serum sHLA-G level could be a diagnostic biomarker in AR patients for clinical severity assessment.

Allergic rhinitis (AR) is an inflammation of nasal mucosa which is characterized by an immunoglobulin E (IgE)-mediated triggering of "nasal congestion, rhinorrhea, sneezing, and itching" in response to specific environmental triggers. The gold standard for AR diagnosis is a clinical history, physical examination findings, and specific IgE level [1]. The main pathological process of AR is mediated by Th2 cells, which secretes IL-4 that induces IgE class switching [2]. Human Leukocyte Antigen G "HLA-G", a non-classical HLA-class Ib molecule with well-known immunomodulatory activities, involving suppression of innate and adaptive immune responses and tolerance induction [3]. Several studies afforded evidence that "sHLA-G serum" levels are elevated in diverse pathological situations, including autoimmune diseases, organ transplantation,

and malignancies [2]. Numerous HLA-G mediated actions have been clear. Although most of them are immunosuppressive, others clarified that the mechanism of action of HLA-G is through induction of "Th2-cytokine" profile by production of "IL-3, IL-4 and IL-10" cytokines [4].

HLA-G is measurable as a soluble form "sHLA-G" in numerous body fluids and originates from the shedding of surface isoforms "sHLA-G1" and/or the releasing of soluble isoforms "HLA-G5, -G6 and -G7" [5]. In physiological conditions, HLA-G forms have dissimilar influence on cytokine production by Th cells: "shed HLA-G1 endorses Th2 shift", while soluble HLA-G5 stimulates tumor necrosis factor (TNF)- α , interferon (IFN)- γ , and IL-10 production [3]. Cell culture studies indicate that coculture of peripheral blood mononuclear cells (PBMC) with HLA-G expressing cells promote a switch towards a Th2 cytokine production,

while stimulation of PBMC with sHLA-G protein provokes an anti-inflammatory milieu due to the IL-10 release. Also, peripheral blood and inflamed tissues contains "HLA-G-positive CD4⁺ CD25 FoxP3⁺ regulatory T cells" [5].

Previous research in AR patients has shown that "serum sHLA-G" levels are: i) markedly elevated compared with controls; ii) raised in seasonal AR during pollen season and outside; and iii) associated with severity of symptoms, therapy, levels of allergen-specific IgE, type of allergy (perennial/seasonal) and immunotherapy [6]. In this study, we evaluated level of the "serum sHLA-G" in AR and in healthy controls and we studied the association between the "serum sHLA-G" and allergen specific IgE levels in AR patients.

Material and Methods

Study design and Subjects

This case control study was carried out at Microbiology and Immunology Department, Faculty of Medicine, Zagazig, Egypt between December 2016 and December 2017. Twenty-five AR patients (14 females and 11 males) were recruited from the ENT unit, Zagazig University Hospitals. AR diagnosis was done according to confirmed principles arranged by Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines 2008 [7]. We excluded upper respiratory infections and patients receiving immunotherapy or taking nasal therapy, antihistamines, corticosteroids, and anti-leukotrienes, during the previous month. Twenty healthy subjects (9 females and 11 males) were enrolled as controls. we collected serum samples from AR patients to measure "serum sHLA-G" and allergen-specific IgE serum levels. The study was approved by the institutional review board (IRB), of the Zagazig faculty of medicine, Egypt and a written consent was obtained by all participants.

Methods

- Clinical parameters

The nasal symptoms of "obstruction, rhinorrhea, sneezing, and itching" at time of collection of blood samples were recorded. The following scale was used to score each symptom: "0 = no symptom", "1 = mild

(symptom was present but was of short duration and not annoying or troublesome", "2 = moderate (symptom was frequently troublesome but did not interfere with either normal daily activity or sleep)", or "3 = severe (symptom was troublesome and interfered with normal daily activity or sleep)". The "total nasal symptom score (TNSS)" was the summation of the scores for the separate nasal symptoms. TNSS values (0–12) were classified as a) mild (0–4), b) moderate (5–8), or c) severe (9–12) [8].

- Analysis of soluble HLA-G level

Serum samples were obtained from all participants. Levels of "serum sHLA-G" were determined by sandwich enzyme-linked immunosorbent assay (ELISA) using sHLA-G ELISA assay kit (MyBioSource, California, USA, Catalog No. MBS267094) according to the manufacturer's instructions. All samples were done in duplicate; and mean absorbance, was determined at a wavelength of 450 nm for each sample. Calibration curves were used to determine the concentration of "serum sHLA-G". The limit of sensitivity was 0.06 U/ml. Results were expressed as concentration of sHLA-G in nanograms per milliliter.

- Allergen-Specific IgE Measurement

Immune blot assay (AllergyScreen test, UK) was used for quantitative determination of serum specific IgE in AR patients against aeroallergen and antioat IgG as positive control with Allergy Screen Panel 2A EGY (MEDIWISS Analytic GmbH, Hanover, Germany) according to the manufacturer's instructions. Briefly, patient's serum was pipetted into a trough of nitrocellulose membrane coated with specific allergens, followed by addition of biotin-coupled anti-human IgE antibody, streptavidin conjugated with alkaline phosphatase, and substrate, in order. The colour intensity of the lines is proportional to the respective allergen specific IgE concentration in the patient's serum. After measurement a printout provides the user with a photo of the membrane, the densitometer curve of the membrane, the classes and the concentration data for each allergen band in IU/ml. The result was stated in IU/ml and classified into six classes (Table 1) reflecting allergen-specific IgE content of the serum according to the manufacturer's instructions. The test was valid if positive control IgE was more than 3.5 IU/ml.

Table 1. Class scores of the specific IgE concentration.

Classes	IgE level (IU/ml)	Allergen-specific IgE
Class I	0.35–0.69	Low
Class II	0.7–3.4	Increased
Class III	3.5–17.4	Significantly increased
Class IV	17.5–49.9	High
Class V	50–100	Very high
Class VI	>100	Extremely high

IgE, immunoglobulin E.

Statistical Analysis

It was achieved by "statistical package of social science (SPSS) version 20" (SPSS Inc., Chicago, IL, USA). Mean and median values with first and third quartiles (interquartile range) were used to express quantitative data while number and percentage used for qualitative data. The nonparametric Kruskal-Wallis rank test was done to assess the analysis of variance between patients' groups. Spearman test was performed to analysis correlations. The accepted level of significance in this work was stated at 0.05, and hence $P < 0.001$ was considered highly significant and $P < 0.05$ was considered significant.

Results

In the present study, both study and control groups were compared as regard to age and sex distribution. The mean age in the AR group was 31.80 ± 5.43 years compared to 27.33 ± 7.99 years, in the control group. In addition, females represented 58.3% of AR group and 45% of the control group. Both AR and control groups were comparable regarding to TNSS. Twenty-five AR patients were symptomatic with a mean TNSS of 5.5 ± 2.15 at time of collection of patient's blood.

Allergen-Specific IgE Measurement

The specific IgE were performed to 10 aeroallergens and the results revealed that the most common allergens were *Alternaria* alternate (44%) and *Dermatophagoides pteronyssinus* (40%) (Figure 1), while cat, feather mix and *Aspergillus niger* were negative. In AR group, mixed grasses and, brich each were positive in six subjects (24%); while sunflower seed was positive in 4 subjects (16%); while *Dermatophagoides farina* and Dog were positive in 2 subjects (8%) (Table 2).

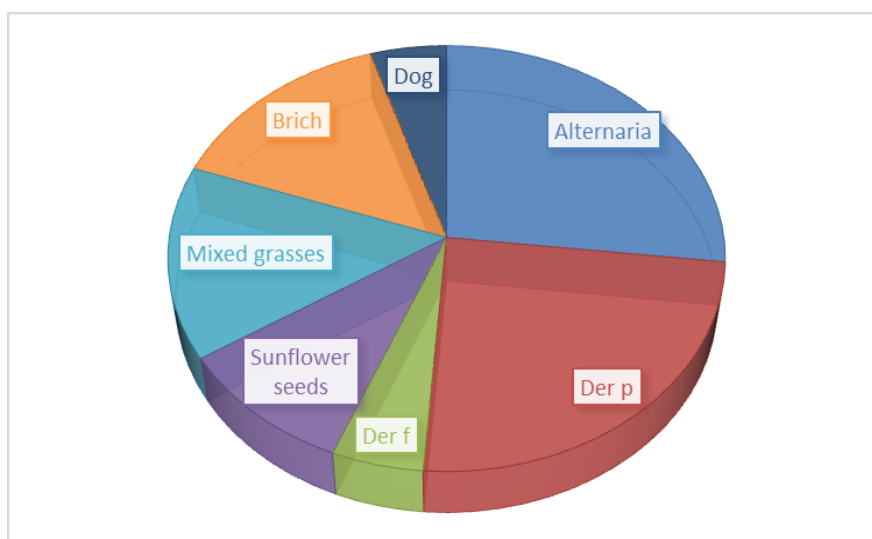


Figure 1. Pie chart showing the number of positive cases of specific IgE to different allergens in AR group.

Table 2. Positive rate of allergen specific IgE in AR group

Allergen	% of positive test
Alternaria	44%
Der p	40%
Der f	8%
Brich	24%
Mixed grasses	24%
Sunflower seed	16%
Dog	8%
Cat	0%
Feather mix	0%
Aspergillus niger	0%

Der p, Dermatophagoides pteronyssinus; Der f, Dermatophagoides **farina**.

sHLA-G level

The median of "serum sHLA-G" levels in control and AR group was 0.140 U/mL and

1.950 U/mL, respectively. AR group had significantly higher serum sHLA-G levels than normal subjects, $P < 0.001$ (Figure 2).

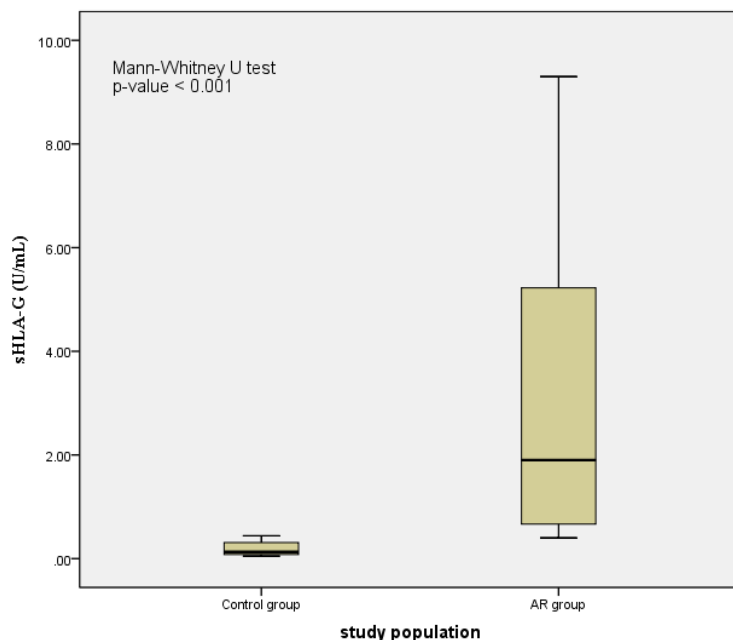


Figure 2. Serum sHLA-G (U/mL) distribution in control and AR groups. Values are represented as medians (horizontal lines), quartiles (25th and 75th percentiles, boxes), and ranges (vertical lines) and p values between groups.

Correlation between "serum sHLA-G" level and allergen-specific IgE Measurement

Figure 3 shows the correlation between "serum sHLA-G" level and allergen-specific IgE level in AR group. There was a highly significant ($P < 0.01$) and positive correlation between "serum sHLA-G" levels and specific IgE levels of *Alternaria alternata* and *Dermatophagoides pteronyssinus* ($r=0.943$, $r=0.775$), respectively. Moreover,

there was a significant ($P < 0.05$) and positive correlation between "serum sHLA-G" levels and specific IgE levels of brich and mixed grasses ($r=0.949$, $r=0.914$), respectively. The scatter plot (Figure 4) shows the correlation between "serum sHLA-G" levels and TNSS in AR group. There was a significant and strong relation between "serum sHLA-G" levels and TNSS ($r=0.785$; $P < 0.001$).

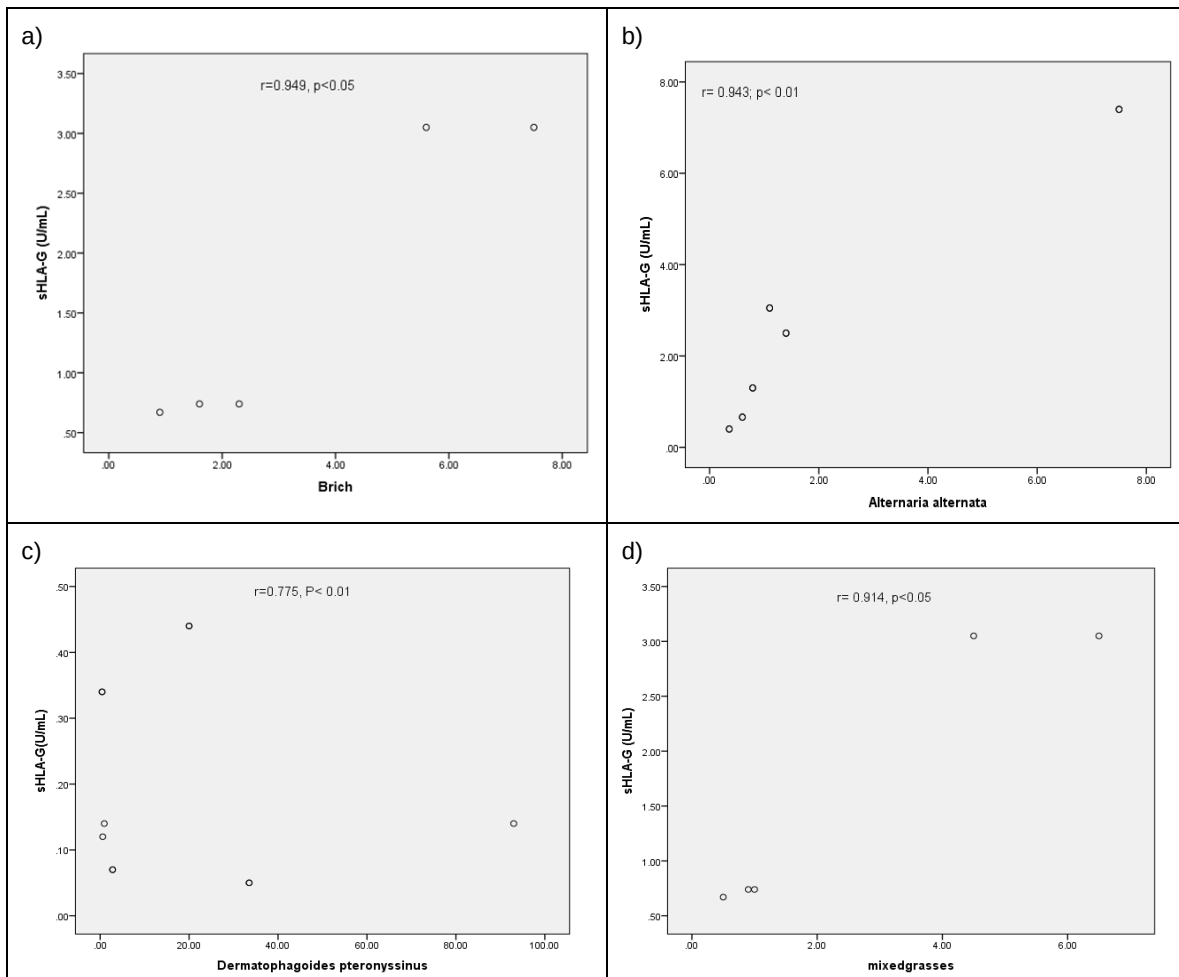


Figure 3. Correlation between serum sHLA-G (U/mL) and serum specific IgE in AR group for a) brich, b) alternara alternate, c) Dermatophagoides pteronyssinus, d) mixed grasses

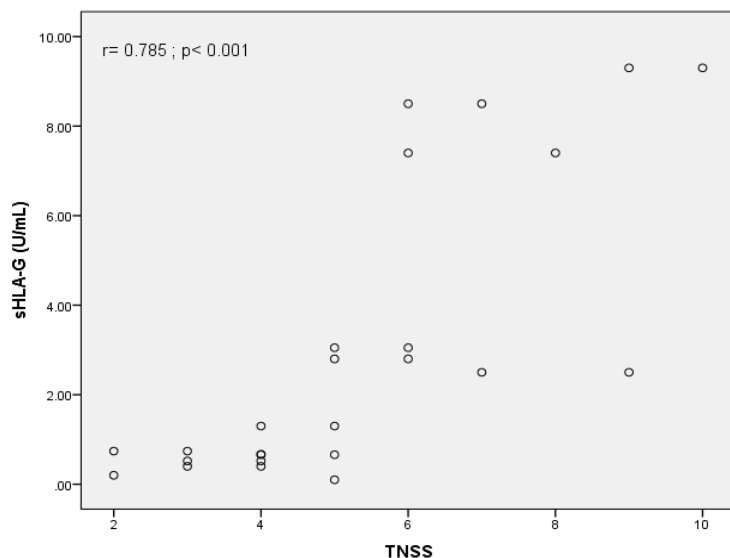


Figure 4. Correlation between Serum sHLA-G (U/mL) levels and severity of symptoms in AR group.

Discussion

Allergic rhinitis is an immunoregulatory disorders with reduced tolerance towards allergens that is characterized by Th2-mediated inflammation. The main pathogenic factors are Th2 derived cytokines, as IL-4, IL-5, and IL13, which induce and augment IgE production [9]. Considering this, there is evidence endorses a central role for HLA-G in tolerance induction and modulation of inflammation [10]. HLA-G may induce tolerogenic DCs and Treg cell production, or function [11].

In the present work, *Alternaria alternate* (44%), *Dermatophagoides pteronyssinus* (40%), mixed grasses (24%), birch (24%), and sunflower seed (16%) were the most common detected allergens. Other detected allergens were just in two subjects. Our results are consistent with the previous literature, where it was reported that, *Alternaria alternate*, *Dermatophagoides pteronyssinus* allergy is strongly implicated

in the pathogenesis of respiratory allergic disease [12,13,14].

The immunoregulatory role of HLA-G have demonstrated in a number of studies that evaluated the expression of sHLA-G in sera from patients with airway allergic diseases as reported by an extensive review [15]. Our study was aimed to evaluate level of the "serum sHLA-G" in AR patients and in healthy control groups and the correlation between "serum sHLA-G" and allergen-specific IgE. Our results displayed a significant rise of "serum sHLA-G" in AR group than in normal subjects. Our findings are in good agreement with previous studies which have shown raised "serum sHLA-G" levels in AR patients with pollen-induced AR considered outside the pollen season [16], during the pollen season [17]. Furthermore, "serum sHLA-G" levels were elevated in patients with perennial AR as a result of perennial allergens including house dust mite (*Dermatophagoides pteronyssinus*), cat and dog dandruff [16].

In our opinion, the elevated HLA-G in AR patients could be either an initiator of inflammation or an immunomodulator in allergic rhinitis. The first is supported by that HLA-G stimulates the Th cytokine balance towards Th2 by promoting the secretion of IL-3, IL-4 and IL-10 but down-modulating the production of IFN- γ and TNF- α . On the other side HLA-G-mediated expression of anti-inflammatory cytokines (e.g. IL-10) will participate in the suppression of the activity of pro-inflammatory cytokines [11]. Furthermore, HLA-G acts at several levels and induces the generation of multiple regulatory cells (HLA-G-acquired Tregs, CD4/8 low suppressor cells, and Tr1 cells), which cooperate, in association with other additional Tregs, in promoting tolerance networks [18]. These observations suggest an immunomodulator role of HLA-G.

There was a highly significant and positive association between "serum sHLA-G" levels and specific IgE levels of *Alternaria alternata* and *Dermatophagoides pteronyssinus*, respectively. Moreover, there was a significant ($P < 0.05$) and positive correlation between "serum sHLA-G" levels and specific IgE levels of birch and mixed grasses, respectively.

Actually, the "immune response to allergens" is characteristically specific to causal allergen. It has been earlier stated that the causal allergen markedly affects clinical symptoms and inflammatory responses. In other words, each separate type of pollen promotes a different score of clinical symptoms and inflammatory response. By the way, it has been previously stated that a more severe immune impairment happened in birch allergy than with grass or *Parietaria* allergy. In this study, which was included only AR patients that are allergic to grasses and/or *Parietaria*, patients with birch allergy

showed the elevated "serum sHLA-G" levels [19]. The limitation of our study is that the AR patients were sensitized to more than one allergen.

Moreover, "serum sHLA-G" levels were studied as biomarkers for assessment of AR severity in association with clinical parameters. Regarding this, we found a significant and strong relation between "serum sHLA-G" levels and TNSS. The same results were obtained by some authors who evaluated clinical severity of AR patients with a "validated visual analogue scale (VAS)" for quantifying the observation of severity of nasal symptoms at the termination of the pollen season [11,17,19]. This matter is very significant as it clarifies the clinical relevance of these findings.

In conclusion, our findings suggest that "serum sHLA-G" level is markedly elevated in AR patients and that "serum sHLA-G" level could be a diagnostic biomarker in AR patients for clinical severity assessment.

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