Original Article

Detection Total Serum Immunoglobulin E (TSIgE) Concentration in Atopic Asthmatic Children with HRV and RSV Infection

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Abstract

Background: Asthma is a chronic lung disease that causes inflammation and narrows the airways. It was considered to be an allergic disease; therefore, immunoglobulin E (IgE) plays a crucial role in this disease because it is highly responsive to allergens such as respiratory syncytial virus (RSV) and human rhinovirus (HRV). Mast cells, T helper 2 cells, and their cytokines play a role in the pathogenesis of atopic asthma. IgE plays a significant role in allergic disease, particularly in asthma. As a result, total serum IgE (TSIgE) level measurement helps with asthma diagnosis and patient status monitoring. **Objectives:** The aims of this study were to measure the TSIgE level in atopic asthma with RSV and HRV, evaluate its relationship to illness severity, and evaluate TSIgE's role in the identification of atopic asthma. **Materials and Methods:** This study included 50 atopic asthmatic children, consisting of 35 males and 15 females, who attended a consulting clinic for respiratory diseases and asthma at Karbala Teaching Hospital for Children. Their age range from 1 to 6 years. Fifty healthy children (32 males and18 females), matched for age and gender with the patients, were selected as control group. The traits and clinical data were collected from consultant, asthmatic children, and/or their parents through a questionnaire. Furthermore, data were obtained from the healthy children and/or their parents during a questionnaire. Whole blood was collected from each child, and the separated sera were used to measure total IgE levels for all samples using the enzyme-linked immunosorbent assay technique. A peripheral blood sample was used to measure the absolute eosinophil count by peripheral blood smear. **Results:** The current results showed a highly significant association between TSIgE concentration in atopic asthmatic patients (275.77 ± 1295) compared to the healthy group (11.153 ± 6.92). Additionally, mean of eosinophils in atopic patients was 6.2 ± 2.5, whereas in healthy group, it was 1.7 ± 0.81 ($P = 0.016$). Moreover, eosinophil count and TSIgE levels were higher in moderate asthma compared to the mild asthma, respectively. HRV and RSV (Immunoglobulin M [IgM] positive) were found at a higher percentage (42.8%) in asthmatic children, with 2 out of 7 (28.5%) and 1 out of 7 (14.3%) children from the age group of 1–2 years ($P = 0.09$), respectively. **Conclusion:** The TSIgE level is important for differentiating between asthmatic and non-asthmatic children. Furthermore, TSIgE levels and eosinophil count aid in diagnosing atopic asthma, especially in children. TSIgE levels are associated with asthma severity.

Keywords: Atopic asthma, ELISA, eosinophil count, TSIgE

Introduction

Immunoglobulin E (IgE) plays a crucial role in allergic disease, particularly in asthma, involving allergens such as human rhinovirus (HRV) and respiratory syncytial virus (RSV) infection. Asthma was a complex multifactorial disease involving environmental factors (the most common trigger in childhood asthma being viral upper respiratory tract infections) and a genetic component. Viruses, such as RSV and HRV, are correlated with 80%–85% of asthma exacerbations in children.[1] It is one of the most rapidly growing disorders among chronic respiratory diseases, affecting people from childhood to old age. It causes recurring periods of wheezing, chest tightness, reversible

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airway obstruction, bronchial hyperresponsiveness, shortness of breath, and coughing. Coughing often occurs at night or early in the morning.[2] Atopy is a condition characterized by inappropriate IgE responses to common environmental antigens or allergens encountered by bronchial mucus. In addition, atopic asthma is triggered by an allergen-induced T helper 2 response, which is correlated with eosinophilia and increased serum IgE levels.[3] Further, serum levels of allergen-specific and total IgE are strongly associated with the degree of sensitization and disease severity in atopic patients. Allergic asthma is defined as asthma associated with sensitization to environmental allergens such as HRV, RSV, and bacterial infections. It was the most common subtype of asthma, accounting for 60%–80% of the entire asthma population. Atopic asthma often begins in childhood and is usually associated with a past history or family history of allergic rhinitis, dermatitis, conjunctivitis, or food and drug allergies.[4] Determination of total serum IgE (TSIgE) levels is used as a screening method for atopy, and an upper limit of 100–180 U/L is generally accepted as a threshold for distinguishing atopics from non-atopics.[5] An allergy diagnosis based on a patient's medical history and/or an IgE definition should be carried out if an allergic trigger is suspected.[6] IgE antibodies were crucial for the treatment of atopic patients. Increased total IgE (total serum Immunoglobulin [TSIgE]) levels promote their survival and prevent them from undergoing apoptosis.[1] There is a complex interaction between IgE and eosinophils. The higher TIgE levels in response to a few prevalent environmental allergens point to an essential role for atopy in the manifestation of asthma in the urban environment. Finally, in atopic asthmatic children, TSIgE was substantially correlated with age, exposure to cigarette smoke, and increased eosinophil count.[7] Therefore, given the aforementioned significance of asthma for both health and the economy, the current study aims to investigate the concentration of IgE in asthmatic children with HRV and RSV infections, as well as some of its influence factors.

Materials and Methods

The current case-control study included 50 asthmatic children, with 35 (70%) males and 15 (30%) females, who attended the outpatient asthma clinic at Karbala Teaching Hospital for Children between February 2022 and June 2022. Diagnosis of asthma was based on clinical and laboratory findings. All children had the American Thoracic Society criteria for asthma.[8] In the current study, asthmatic children aged between 1 and 6 years old were included. The healthy control group consisted of 50 children, comprising 32 (64%) males and 18 (36%) females, with the same ages and gender distribution as the patients. These children had no history of allergies or family history of asthma and were selected from the local community. All participants underwent full history taking, clinical examination, and laboratory investigations, including TSIgE, IgM concentration of HRV and RSV, for 50 atopic and 50 control subjects. These parameters were determined using enzyme-linked immunosorbent assay (ELISA).

Collection of samples

Four milliliters of peripheral blood were collected from each participant in the current study via vein puncture, under complete aseptic conditions. The blood was then dispensed into a 2 mL ethylene diamine tetracetic acid tube for hematological analysis, including total and differential white blood cell counts, which were measured using Sysmex XN-350 five differential automated hematology analyzer (Sysmex, Kobe, Japan). In addition, 2 mL of blood were placed into plain tube and allowed to clot. The serum was separated by centrifugation at 3000 RPM for 15 min at room temperature. To measure TIgE levels, repeated freezing and thawing of serum samples were avoided. The measurements were performed using the BioTek ELx 800 automated immunoassay analyzer (USA) with the AccuBind TIgE ELISA kit (LOT No. 25K1D) from Monobind, Inc, CA, United States. Additionally, ELISA kits for HRV-IgM and RSV-IgM were used for the qualitative determination of HRV and RSV-IgM in serum from atopic asthmatic children.

Data analysis

The data were subjected to statistical analysis using the Statistical Package for the Social Sciences (SPSS) version 21 for Windows (GraphPad Software, San Diego, California). One-way analysis of variance (ANOVA) was used for comparisons of means, and significance differences were accepted as $P < 0.05$. The data were presented as mean ± SD.

Ethical approval

Approval for the study protocol was granted by Karbala Health Directorate's ethical committee. The study was conducted in accordance with ethical principles originating from the Declaration of Helsinki. It was carried out with verbal and written approval from the patients before samples were taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee under document number 074 dated March 23, 2022.

Results

The total age range of asthmatic children in the current study was between 1 and 6 years, distributed into five age groups: 1–2, 2.1–3, 3.1–4, 4.1–5, and 5.1–6 years. The present results revealed that the disease was more frequent in 5.1–6 years (44.0%) in atopic asthmatic children [Figure 1]. The current results showed demographic and biochemical profile of atopic asthmatics and non-asthmatic (healthy) children. The children with atopic

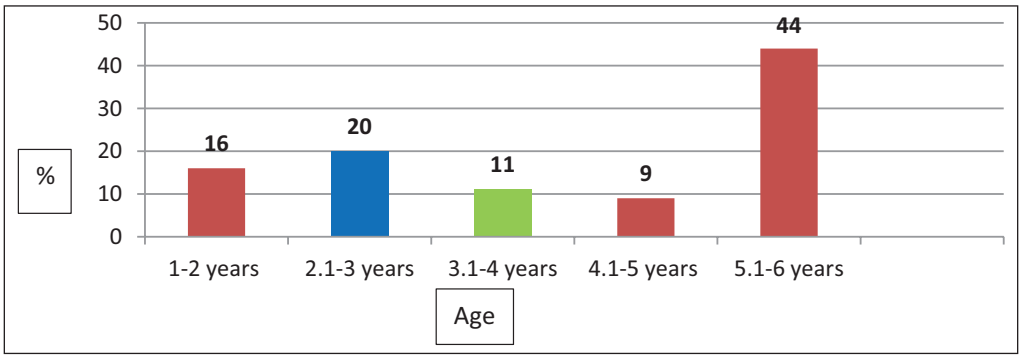

Figure 1: Age distribution of atopic asthmatic children

Table 1: Allergic characteristics of asthmatic group and controls

Demographic and biochemical profile		Number of asthmatic children	%	Control children	%	*P* value
Age, mean ± SD		4.4 ± 1.66		4.0 ± 1.55		0.057
Gender	Male	35	70	32	64	0.06*
	Female	15	30	18	36	
% Eosinophil count		6.2 ± 2.5		1.7 ± 0.81		0.016
TSIgE (IU/mL)		275.77 ± 1295		11.153 ± 6.92		0.002**
Family history of asthma/allergic diseases	Yes	44	88	0	0	0.000**
	No	6	12	50	100	0.011

**Highly significant by one-way ANOVA test ($P < 0.0.05$)

%: percentage ; TSIgE: total serum Immunoglobulin; SD: slandered deviation

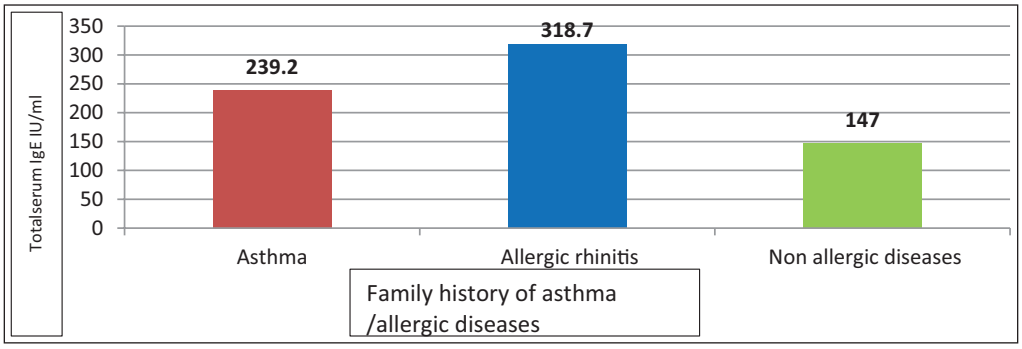

Figure 2: Difference in the mean TSIgE levels between family histories of allergic disease

asthma exhibited clinical symptoms and a high level of TIgE, while the control group had a normal TIgE concentration, as shown in Table 1.

These results showed no significant associations of age, height, weight, and body mass index, while significant differences for gender between the patients and controls were found.

There was a highly significant difference of TIgE level, percentage of eosinophil count, and family history of asthma between them, as shown in Table 1. The current result observed that 50 (100%) of atopic asthmatic patients had high TSIgE. The mean TSIgE levels in atopic asthmatic children were 275.77 ± 1295 IU/mL, while for healthy individuals, mean TSIgE levels were 11.153 ± 6.92 IU/mL. On the other hand, the mean percentage of eosinophil count was 6.2 ± 2.5 in the asthma group and 1.7 ± 0.81 in the control group, as detailed in Table 1. Moreover, the mean TIgE differs between patients with a family history of asthma compared to those with allergic rhinitis, as shown in Figure 2.

In addition, asthma severity among children in the current study was classified as mild and moderate. The number of patients with mild asthma was higher than the number of patients with moderate asthma, and the mean TIgE levels in moderate atopic asthmatic children were higher compared to those with mild asthma, as shown in Figure 3.

A total of 100 blood samples, including 50 samples from suspected viral (HRV and RSV) infected

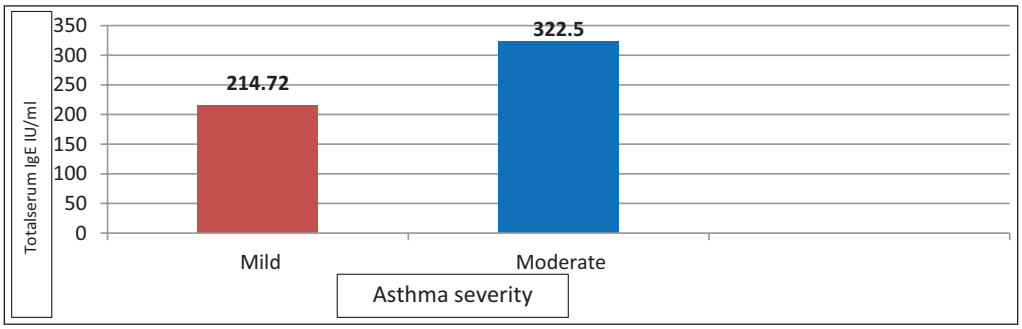

Figure 3: The mean TSIgE levels between mild and moderate asthmatic children

Table 2: Identification of HRV and RSV in asthmatic children and control (IgM positive) by ELISA

Comparison groups	*N*	Number of HRV	%	Number of RSV	%	Total	%	*P* value
Atopic asthmatic patients	50	10	20	1	2	11	22	0.437
Control	50	0	0	0	0	0	0	
Total	100	10	10	1	1	11	11	
P value		0.250		0.362				

Nonsignificant by one-way ANOVA test ($P > 0.05$)

HRV: human rhinovirus, RSV: respiratory syncytial virus, ELISA: enzyme-linked immunosorbent assay

Table 3: Association of gender with HRV and RSV infection in asthmatics and control

Comparison groups	*N*	No. +ve IgM HRV				*P* value (S)	No. +ve IgM RSV						*P* value
		Male	%	Female	%		Male	%	Female	%	Total	%	
Atopic asthmatic patients	50	8	16	2	4	0.090*	1	2	0	0	11	22	0.500
Non-asthmatics	50	0	0	0	0		0	0	0	0	0	0	
Total	100	10			10		1			1	11	11	

Nonsignificant by one-way ANOVA test ($P < 0.05$)

No: number, %: percentage, TSIgE: total serum Immunoglobulin, +ve: positive, HRV: human rhinovirus, RSV: respiratory syncytial virus

Table 4: Association of risk factors with HRV and RSV infection in asthmatics and non-asthmatic children

Comparison groups			HRV				*P* value	RSV					*P* value (S)
			+ve IgM		−ve IgM			+ve IgM		−ve IgM			
			N	%	*N*	%		*N*	%	*N*	%	Total	
Atopic	Residence	Urban	7	17.1	34	82.9	0.031	1	2.4	40	97.8	41	0.000
		Rural	3	33.3	6	66.7		0	0	9	100	9	
	Passive smoking	+ve	8	25.8	23	74.2	0.041	1	3.2	30	96.8	31	0.04
		−ve	2	10.5	17	89.5		0	0	19	100	19	
		−ve	0	0	16	100		3	17.6	14	82.4	17	

Nonsignificant by one-way ANOVA test ($P < 0.05$)

HRV: human rhinovirus, RSV: respiratory syncytial virus

asthmatic patients and 50 samples from controls, were used in ELISA test. Among these, 10 samples (20%) from atopic patients tested positive (+ve IgM) for HRV, and 1 sample (2.0%) tested positive for RSV. However, all samples from healthy control group tested negative result for this test, as illustrated in Table 2. Additionally, most asthmatic patients in the present study infected with HRV and RSV were males compared to females, as shown in Table 3.

Table 4 presents data showing that 7 out of 50 (14%) atopic asthmatic children with acute HRV infection were living in the urban areas, while 3 (6%) were in rural areas. For RSV, 1 (2%) was from an urban area and none from a rural area. However, it's noteworthy that residence showed an association with acute HRV and RSV infection in asthmatic children. Furthermore, there were significant associations between residence and passive smoking with acute HRV and RSV infection in asthmatic patients, as shown in Table 4.

Discussion

The present results demonstrate that the frequency of atopic asthma in children increased with age, the age group of 5.1–6 years being at higher risk for developing this disease, as shown in Figure 1. Reference [9] reported that 43% and 30% of asthmatic children belonged to the age groups of <5 and 5–10years, respectively, with increased TSIgE (≥100 IU/mL). The current study showed increased TSIgE in atopic asthmatic children compared to the control group. In addition, there was a highly significant difference between asthmatic and healthy control group in TIgE level. Some studies on IgE concentration have found that total IgE levels are significantly increased in asthmatic patients, which supports the present data.[10,11] Furthermore, atopic asthma is characterized by impaired spontaneous release of interferon (IFN)-gamma and increased production of interleukin (IL)-4, which correlates with the magnitude of eosinophilic inflammation. References [12] and [13] showed that out of a total of 56 asthmatic patients, 21 (37.5%) had allergic asthma. Most patients with allergic asthma had high TIgE levels (233.5 KU/L), compared to the control group (14.25 KU/L). Additionally, serum eosinophil counts were higher in allergic asthma patients (0.271×10^9/L) compared to the control group (0.095×10^9/L). Reference [14] showed that the mean TIgE levels and absolute eosinophil count were 221.07 ± 774.55 IU/mL and 214 ± 360/µL, respectively. On the other hand, as shown in Figure 1, there was an effect of family history of asthma/allergic diseases on TIgE levels. Reference [15] indicates that 100% of atopic asthmatic patients have a family history of allergies. However, there was no significant difference between asthmatic and allergic rhinitis patients ($P = 0.270$). The current result agrees with a local study, which revealed that 48.7% of asthmatic children had positive IgE screening test and 51.3% of them had negative IgE screening test.[9] The current results showed high TIgE in all atopic asthmatic children (100%). Furthermore, there was a highly significant difference between atopic asthmatic and healthy control group children in IgE concentration ($P = 0.000$). The recent study revealed that serum level of TIgE was significantly higher in asthmatic children (275.77 ± 1295 IU/mL) compared to the control group (11.153 ± 6.92 IU/mL). In addition, the current results are consistent with the study by Ref. [16], which found elevated TIgE levels in atopic asthmatic children compared to the control group, indicating a strong positive relationship between TSIgE levels and asthma in children. TIgE was elevated in multiple allergic diseases and is considered a good predictor of atopy.[17] The present results revealed elevated levels of TIgE in the following age groups: 1–2 years (14%), 2.1–3 years (18%),3.1–4 years (10%), 4.1–5years (10%), and 5.1–6 years (48%). The mean serum IgE value of bronchial asthma patients was 374.3 IU/mL, which was higher than that of the general population.[18] The current results indicate that the female gender was not predominant in both elevated IgE concentration in atopic and normal IgE level non-asthmatic children compared to male. Additionally, there was a nonsignificant association between IgE level and gender. This result may be attributed to previous reports indicating that atopic asthma in children is more prevalent in males than in females. In general, the levels of TIgE increased as the severity of asthma increased. In the current study, passive smoking was positive in 62% of cases, whereas 38% were negative, as shown in Table 2. These findings align with those of Ref. [19], which demonstrated that exposure to smoking among atopic asthmatic children was the most environmental factor stimulating atopic asthma and elevating TIgE concentration. The same study found that 60% of atopic asthmatic children exposed to smoking had elevated TIgE levels, while only 40% of them had normal values of TIgE concentration. The current study revealed that the percentage of eosinophils count was high (6.2 ± 2.5), and the levels of IgE were elevated in atopic asthmatic children compared to healthy children in the normal IgE group, showing a 100% increase. There was a highly significant difference ($P < 0.000$). The present result agrees with Ref. [20], who observed that there was a strong relationship between total eosinophil count and TIgE in atopic asthma.

Many studies have concluded that there is a relationship between living in urban areas and elevated TIgE concentrations, as shown in Ref. [21] Moreover, this study revealed an increase in TIgE levels among atopic asthmatic children living in urban areas compared to those living in rural areas. The mean difference in TIgE levels between mild and moderate asthmatics was 214.7 and 322.5 IU/mL, respectively. However, the previous investigation revealed a TSIgE level of 297.773 IU/mL in moderate asthmatic children, while mild atopic asthmatic children had a TSIgE level of 219.761 IU/mL. Moreover, severe asthma patients exhibited a higher mean IgE level (280.2 IU/mL) than patients with moderate (145.8 IU/mL) or mild (137.8 IU/mL) asthma. Thus, IgE levels increased with asthma severity.[6,22] The high yield of ELISA IgM positivity for HRV and RSV in first years of children's age could be explained by the fact that most acute HRV and RSV infections occur in the first 6 years of life, as shown in Table 4. The present result is lower than that reported by Ref. [23] in Egypt, which was 16.4%. This difference might be attributed to the smaller sample size (427 children) enrolled in the Egyptian study, all of whom were under 5 years of age, and to the fact that the diagnosis was established using immunofluorescent-antibody test of the nasal aspirate. In regard to age as a risk factor for acute HRV and RSV infection in asthmatic children, the current result concluded that there is no significant association between age and acute HRV and RSV infection in children aged 1–6 years, as

shown in Table 4. In contrast, Ref. [24] reported that 8 out of 31 (25.8%) asthmatic children aged from 7 months to 12 years experienced HRV infections, highlighting the significance of HRVs as triggers of asthma exacerbations among Egyptian children. Additionally, 8 (16%) of asthmatic children infected with HRV were males, while 2 (4%) were females. In contrast, RSV infection affected only 1 (2%) male, as shown in Table 4. However, according to Ref. [25], 35 (70%) of the cases were males, while 15 (30%) were females. The present result, which was lower than that reported by Ref. [23] in Egypt (16.4%), may be attributed to the smaller sample size (427 children enrolled in the Egyptian study, all under 5 years of age) and the fact that the diagnosis was established by IFA of the nasal aspirate. When considering age as a risk factor for acute HRV and RSV infection in asthmatic children, it is noteworthy that Ref. [25] reported 8 out of 31 (25.8%) asthmatic children aged from 7 months to 12 years were affected, indicating that HRVs are significant triggers for exacerbations among Egyptian children. Additionally, bacterial infections have been shown to affect children,[26,27] serving as triggers for asthma. Additionally, 8 (16%) of asthmatic children infected with HRV were males, and 2 (4%) were females, while RSV affected only 1 (2%) in male, as shown in Table 5. However, Ref. [25] reported that 35 (70%) of the cases were males, and 15 (30%) were females.

Conclusions

IgE plays an important role in the diagnosis of asthma, and there are factors that affect IgE levels in asthmatic children, such as age, eosinophil count, and asthma severity.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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