

Significance of total and specific IgE in asthma of Iraqi adult patients

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ABSTRACT

A case-control retrospective study was conducted during January – June 2019 on 104 patients with asthma (50 females and 54 males) and 111 non-asthmatic healthy subjects matched patients for gender (54 females and 57 males) to assess role of total and specific IgE serum levels in patho-physiology of disease using ELISA and multiplex immunoblot kits. The mean age \pm standard deviation of asthma patients was 37.9 ± 12.1 years, while it was lower among control (35.9 ± 10.5 years), but the difference was not significant. Males and females were similarly distributed among asthma cases (48.1 and 51.9%, respectively) and control (48.6 and 51.4%, respectively). Most of asthma cases were observed to have mild disease (49%), followed by moderate and severe asthma (93.4 and 11.5%, respectively). It was also observed that 51.9% of patients had a family history of asthma. With respect to allergen type, 21.2% of asthma cases were seropositive for mixed types of allergens. Median level of total IgE was significantly ($p < 0.001$) elevated in sera of asthma cases (204.1 ng/ml) compared to control subjects (163.3 ng/ml). The level positively paralleled the severity of disease. The highest level was observed in severe cases (244.9ng/ml, while the lowest level was recorded in mild cases (189.7 ng/ml). The level of specific IgE showed some variations among asthma cases distributed according to the type of allergen, the highest median was recorded in seronegative cases (215.2 ng/ml), while a lowest level was observed in cases seropositive for animal dander (189.3 ng/ml). Logistic regression analysis revealed that total asthma patients, as well as male and female cases were at an increased risk to develop asthma episodes due to elevated serum level of IgE. ROC analysis demonstrated that elevated serum level of IgE occupied a significant ($p = 0.001$) area under curve (AUC), which was 0.828. In conclusion, the present study confirms the significant role of total and specific IgE in patho-physiology of bronchial asthma and its correlation with disease severity and allergen type in adults

Introduction

Asthma is described as a disease of the airways characterized by chronic inflammation that is linked to hyper-responsiveness in the airways due to exposure to allergic antigens. Consequently, recurrent episodes of wheezing, coughing, chest tightness and breathlessness are triggered [1]. The disease inflammatory response is markedly mediated by interleukins (ILs) belongs to the T-helper 2 (Th2) lymphocyte, which include IL-4, IL-5 and IL-13. Patho-physiologically, abundant eosinophilia and

elevated levels of immunoglobulin E (IgE) are prominently associated with the asthma airway inflammation [2]. It has addressed that IgE is a key immunoglobulin in triggering the inflammatory response in asthmatic patients, as well as asthma evolution and chronicity [3]. It mediates its effects through binding to high-affinity cell surface receptors expressed on mast cells and basophils (Fc ϵ RI), as well as low-affinity receptors present on B cells (Fc ϵ RII) [4].

The most important cells on initial exposure to allergen are dendritic cells (antigen-presenting cells), which have the ability to sensitize naïve T lymphocytes and orchestrate their progression into Th2 (T-helper 2) cells specific for the allergen. Consequently, the production of inflammatory cytokines (IL-4 and IL-13) is induced, and B lymphocytes are triggered to synthesis IgE specific for allergen [5]. The synthesized IgE binds cell-surface receptor on mast cells and basophils (FcεRI). Such binding sensitizes these cells to the allergen, and on repeated exposure, the membrane-bound IgE is cross-linked and consequently basophils and mast cells are degranulated and release allergy mediators (for instance, histamine, platelet-activating factors and leukotrienes) in the early allergic immune [6]. The early response is characterized by marked synthesis of IL-3, IL-4, IL-5 and IL-13, as well as the chemokine ligand-5 and GM-CSF (granulocyte-macrophage colony-stimulating factor). These mediators recruit neutrophils, eosinophils, basophils, macrophages and T lymphocytes to the site of inflammation in the late allergic response [7]. Due to the significance of IgE in patho-physiology of asthma, the present study total and specific IgE serum level in Iraqi asthmatic patients. Correlations with gender, family history, disease severity and types of allergens were also determined.

Materials and Methods

Patients and control

A case-control retrospective study was conducted during January – June 2019 on 104 patients with asthma (50 females and 54 males). The protocol of study was approved by the Ethics Committee at the Iraqi Ministry of Health and Environment. The patients were recruited from the laboratory of Allergy and Immunology at the Allergy Specialized Center (Alresafa, Baghdad, Iraq). The diagnosis of asthma was carried out by consultants at the center. It was based on the guidelines of GINA (Global Initiative for Asthma) [8]. These guidelines were also considered to define the asthma severity (mild, moderate and severe disease). Patients with chronic respiratory tract infections were not included in the study. Family history of asthma was also determined in patients. It was defined as “yes” if one family member experienced the asthma inclusion criteria (sibs, parents or grandparents). The control group included 111 non-asthmatic healthy subjects matched patients for gender (54 females and 57 males).

Measurement of total and specific IgE

From each participating subject, 3 mL of peripheral blood was obtained and dispensed into a plain tube. After clotting at room temperature, serum was obtained by centrifugation in a temperature-controlled centrifuge. The obtained sera were frozen at -20 °C until assessment of total and specific IgE. Total IgE (tIgE) serum level was explored using sandwich-enzyme-linked immunosorbent assay (ELISA) kit (Sun long Biotech Company, China).

The normal range of total IgE in serum of health males and females was 18.8 – 222.7 and 29.9 – 226.4 ng/ml, respectively. Values of 137.3 – 374.8 ng/ml were considered a positive range of tIgE in serum of male and female asthmatic patients.

Specific IgE (sIgE) was also qualitatively determined (negative or positive) using multiplex immunoblot kit (Euroimmun, Germany). The kit detects a profile of 25 inhalation allergens. For simplicity, four major allergen groups were considered and included molds, grasses, animal dander and mites). In both cases, instructions of manufacture were followed.

Statistical analyses

Total serum level was tested for normal distribution using Kolmogorov-Smirnov and Shapiro-Wilk tests. The normal distribution was not ascertained; therefore, the IgE level was presented as median and range. Significant differences between medians were statistically tested by the non-parametric test; Mann–Whitney U test (for comparison between two medians) or Kruskal–Wallis test (for comparison between more than two medians). In the case of age, the parametric student T test was applied to assess significant levels. Pearson Chi-square test or two-tailed Fisher exact test was used to assess significant differences between frequencies. Logistic regression analysis was applied to determine odds ratio (OR) and its 95% confidence interval (CI). Receiver operating characteristic (ROC) analysis was employed to assess the area under curve (AUC), sensitivity, sensitivity and cut-off point of IgE. A probability (p-value) ≤ 0.05 was considered significant. The statistical package SPSS version 25.0 was employed to carry out these analyses. To determine the statistical validity of the enrolled samples, their numbers were tabulated in the datasheet of the software G*Power to estimate power of sample size at an alpha level of 0.05 [9].

Results

Power of sample size

The estimated powers of sample size were 0.94 and 0.95 for patients and control, respectively. The accepted power of sample size is 0.80; therefore, the sample size of patients and control was suggested to be adequate from the view point of statistics.

Baseline characteristics of patients and control

Asthma patients were characterized in terms of age and gender. The patients were further characterized according to severity of disease, family history of asthma and allergen types. The mean age ± standard deviation (SD) of asthma patients was 37.9 ± 12.1 years, while the mean age was lower among control (35.9 ± 10.5 years), but the difference was not significant ($p = 0.190$) (Figure 1).

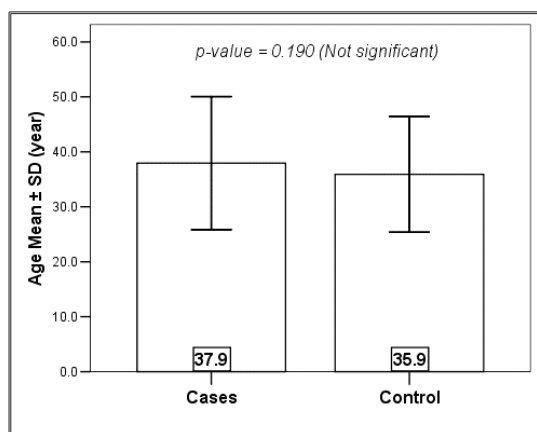


Figure 1: Mean ± SD among asthma cases and control.

Males and females were similarly distributed among asthma cases (48.1 and 51.9%, respectively) and control (48.6 and 51.4%, respectively), and there was no significant difference between their frequencies ($p = 0.933$) (Table 1).

Table 1: Frequencies of males and females among asthma cases and control.

Gender	Asthma cases (N = 104)		Control (N = 111)	
	N	%	N	%
Male	50	48.1	54	48.6
Female	54	51.9	57	51.4

Pearson's chi-square test = 0.009; D.F. = 1; $p = 0.933$ (Not significant).

Mean age of male cases was higher than that of female cases (39.4 ± 12.7 vs. 36.6 ± 11.5 year). Age of male cases was also higher than that of control males (39.4 ± 12.7 vs. 35.2 ± 12.0 year); however, the differences attended no significant ($p > 0.05$) level (Table 2).

Table 2: Mean age of asthma cases and control distributed according to gender.

Gender	Age mean ± SD (year)		p
	Asthma cases (N = 104)	Control (N = 111)	
Male	39.4 ± 12.7	35.2 ± 12.0	0.083 (NS)
Female	36.6 ± 11.5	36.6 ± 9.0	0.999 (NS)
p-value	0.878 (NS)	0.492 (NS)	

NS: Not significant ($p > 0.05$); SD: Standard deviation.

Most of asthma cases were observed to have mild disease (49%), followed by moderate and severe asthma (39.4 and 11.5%, respectively) (Figure 2).

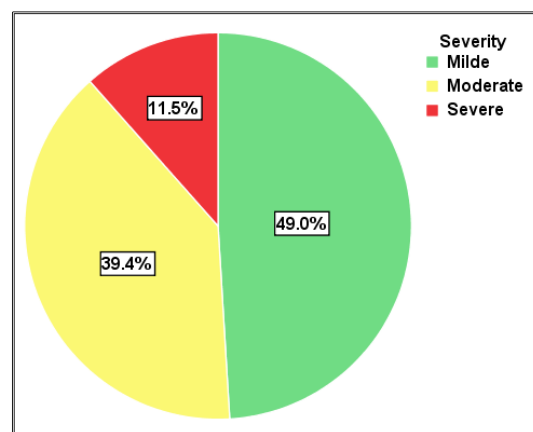


Fig. 2: Asthma cases distributed according to severity of disease.

Family history of disease was defined as the presence of asthma in sibs, parents or grandparents. It was observed that 51.9% of patients had a family history of asthma, while 48.1% represented sporadic cases (i.e. no family history of asthma), as shown in (Figure3).

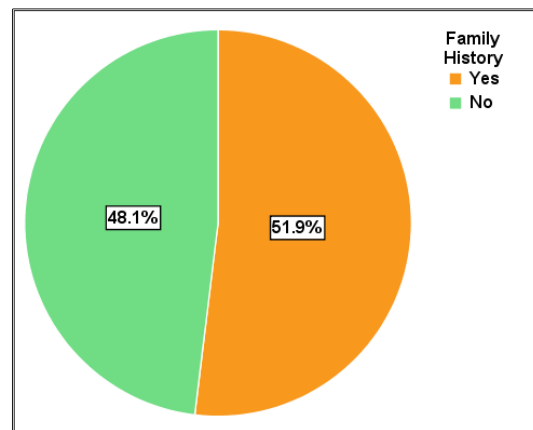


Fig. 3: Asthma cases distributed according to family history of disease.

With respect to allergen type, it was observed that 21.2% of asthma cases were seropositive for mixed types of allergens. However, most of patients were seropositive for mites, followed by molds and grasses (7.7% each) and animal dander (1.9%). It is worth to mention that 28.8% of patients were seronegative for the allergens provided with the kit (Figure 4).

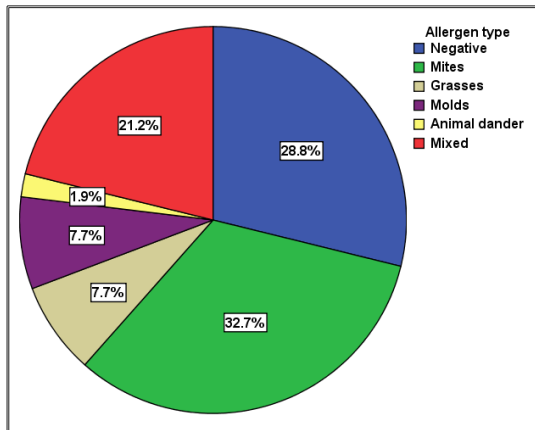


Fig. 4: Asthma cases distributed according to the type of allergen that were seropositive for it.

Total and specific serum level of IgE

Serum level of IgE was significantly deviated from normal distribution (Table 3). Accordingly, the level was given as median and range rather than mean ± SD. Thus, significant differences between medians were assessed by either Mann–Whitney *U* test (for comparison between two medians) or Kruskal–Wallis test (for comparison between more than two medians).

Table 3: Normality testing of total IgE serum level

Group	Test Total IgE Normal Distribution					
	Kolmogorov-Smirnov			Shapiro-Wilk		
	Statistic	D.F.	<i>p</i>	Statistic	D.F.	<i>p</i>
Cases	0.179	104	0.001	0.771	104	0.001
Control	0.175	111	0.001	0.928	111	0.001

D.F.: Degrees of freedom

Median level of tIgE was significantly ($p < 0.001$) elevated in sera of asthma cases (204.1 ng/ml; range: 137.3 – 374.8) compared to control subjects (204.1 163.3 ng/ml; range: 18.8 – 226.4) (Figure 5).

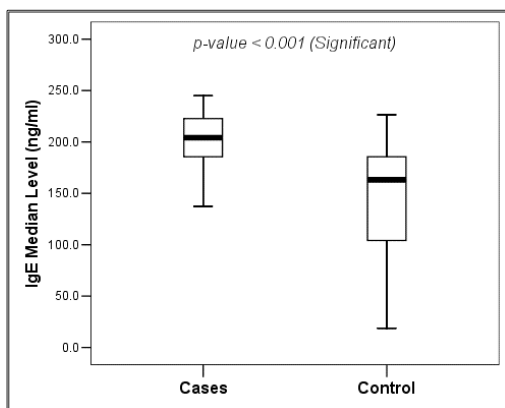


Fig. 5: Box-plot presentation of IgE level among asthma cases and control.

There was a significant ($p = 0.026$) difference between the median serum level of tIgE between male and female patients (218.9 and 194.8 ng/ml, respectively; range: 137.3 – 374.8). Such difference was not observed between medians of control males (167.0 ng/ml; range: 29.9 – 226.4) and control females (159.6 ng/ml; range: 18.8 – 222.7). However, comparing asthma cases (males and females) to the

corresponding control groups revealed significantly ($p < 0.001$) increased median level of tIgE (Table 4).

Table 4: Median serum level of tIgE among asthma cases and control distributed according to gender.

Gender	IgE Median; ng/ml		<i>p</i>
	Asthma cases (N = 104)	Control (N = 111)	
Male	218.9	159.6	< 0.001
Female	194.8	167.0	< 0.001
<i>p</i> -value	0.026	0.818 (NS)	

NS: Not significant (p -value > 0.05).

Median level of tIgE among asthma cases positively paralleled the severity of disease. The highest level was observed in severe cases (244.9 ng/ml; range: 137.3 – 374.8), while the lowest level was recorded in mild cases (189.7 ng/ml; range: 137.3 – 378), and the difference was significant ($p < 0.001$) (Figure 6).

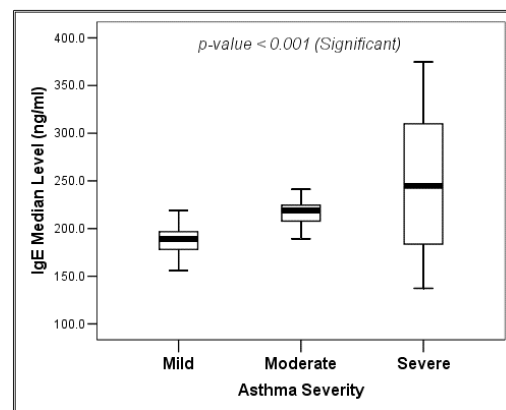


Fig. 6: Box-plot presentation of IgE level among asthma cases distributed according to severity of disease.

Cases with family history of asthma showed non-significantly elevated median of tIgE (207.8 ng/ml; range: 155.9 – 374.8) compared to cases without family history of asthma (200.4 ng/ml; range: 135.5 – 363.9; $p = 0.374$) (Figure 7). However, both ranges were within the positive range of sIgE in asthmatic patients (137.3 – 374.8 ng/ml).

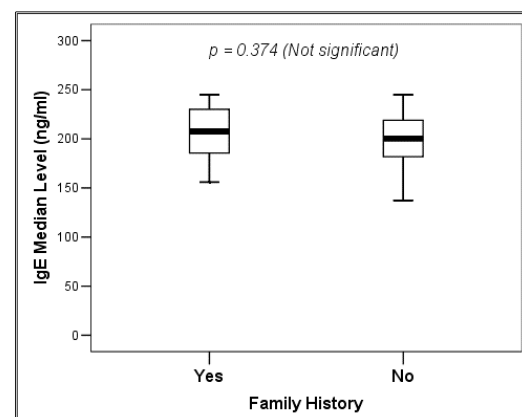


Fig. 7: Box-plot presentation of tIgE level among asthma cases distributed according to family history of disease.

Median level of sIgE showed some variations among asthma cases distributed according to the type of allergen that was suggested to provoke the disease. The highest median was recorded in seronegative

cases (215.2 ng/ml; range: 137.3 – 374.8), while a lowest level was observed in cases seropositive for animal dander (189.3 ng/ml; range: 155.9 – 222.7). However, these differences were not significant ($p = 0.308$) (Figure 8).

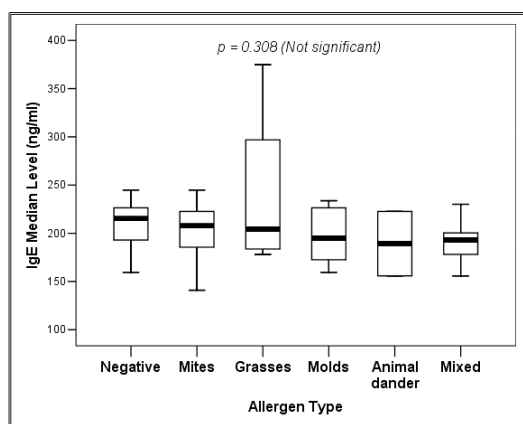


Fig. 8: Box-plot presentation of sIgE level among asthma cases distributed according to types of allergens.

Logistic regression analysis of IgE

Logistic regression analysis revealed that total asthma patients, as well as male and female cases were at an increased risk to develop asthma episodes due to elevated serum level of IgE. The OR of the three evaluations was 1.04, and scored a significant difference ($p = 0.001$). Distributing patients according to severity of asthma demonstrated that cases with a severe disease were significantly at a greater asthma risk due to increased IgE level versus mild disease (OR = 1.002; $p = 0.005$). With respect to family history of asthma, both groups (positive and negative family history) at a similar risk and no significant OR value was recorded. Finally, carrying out the regression analysis in patients with different inhalation allergens versus seronegative patients (reference category) demonstrated a significant risk ($p = 0.018$) in asthma cases seropositive for mixed allergens (Table 5).

Table 5: Logistic regression analysis of IgE serum level among asthma cases and control.

Group	Reference category	OR	95% CI	p
Cases	Control	1.04	1.03 – 1.05	0.001
Male cases	Control males	1.04	1.03 – 1.06	0.001
Female cases	Control females	1.04	1.02 – 1.05	0.001
Moderate severity	Mild severity	1.01	1.00 – 1.02	0.076
Severe severity		1.02	1.01 – 1.03	0.005
Family history (+ve)	Family history (-ve)	1.01	1.00 – 1.01	0.276
Allergen type				
Mites	Negative	0.99	0.98 – 1.00	0.121
Grasses		1.00	0.99 – 1.02	0.552
Molds		0.98	0.96 – 1.01	0.202
Animal dander		0.98	0.93 – 1.03	0.326
Mixed		0.98	0.96 – 1.00	0.018

Receiver operating characteristic (ROC) Analysis

To assess the diagnostic significance of IgE elevated level among asthma patients, ROC analysis was carried out. The analysis revealed that elevated serum level of IgE occupied a significant area under curve (AUC), which was 0.828 ($p = 0.001$). At a cut-off value of 183.7 ng/ml for tIgE level, the sensitivity and specificity were 76.0 and 74%, respectively (Figure 9).

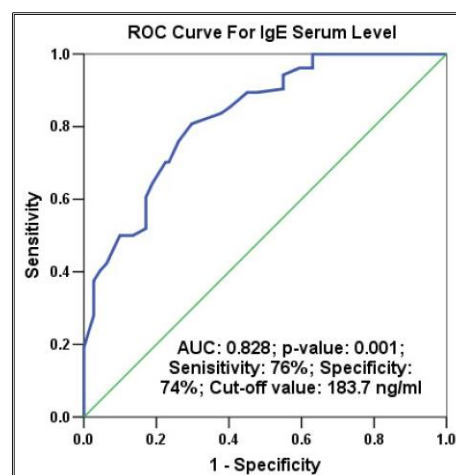


Fig. 9: Receiver operating characteristic (ROC) analysis of IgE serum level among asthma patients showing area under curve (AUC), p-value, sensitivity, specificity and cut-off value.

Discussion

As expected, the total IgE level was significantly elevated in asthma patients compared to healthy, and these results are consistent with the findings of previous related studies [3, 4, 10, 11]. The median level of IgE was significantly different among male and female cases. Such difference was not observed in control males and females. Such gender-related difference might be influenced by the environment of homes. For instance, unvented household gas appliances are suggested to influence symptoms of allergy in atopic females more than in atopic males [12]. Further, it is expected that females are often exposed to indoor allergens more than males. Female-gender associated factors (i.e. hormonal) may also account for an increase asthma risk among females [13]. Studies demonstrated that some female sex hormones are potential proinflammatory mediators and associated with an increased atopy-risk. In this context it has been demonstrated that estrogen signaling promoted allergen-mediated type 2 airway inflammation, while androgen signaling attenuated the inflammation [14]. Further, menstrual cycle has also been associated with susceptibility to asthma, and asthma attacks in women might be triggered by menstruation. About 30–40% of women with asthmatic episodes showed worsening of asthma symptoms during the pre- or peri-menstrual phase of their menstrual cycle [15].

The highest level of IgE was observed in severe cases, while a lowest level was recorded in mild cases and the difference was significant. Sandeep and colleagues found that levels of IgE increased as the severity of asthma increased [16]. In contrast, Davila showed increase of total serum IgE in both mild and moderate asthma, whereas no differences between them [17]. The increase of IgE levels in mild and moderate asthma because IgE binds to receptors on effector cells (basophils and mast cells) with high

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affinity, and this is accomplished with cross-linking IgE by allergens. As a consequence, inflammatory cascade of proinflammatory mediators are released and exaggerate the symptoms of acute and chronic asthma [7]. Borish et al. demonstrated further that high concentrations of total IgE are more likely linked with moderate and severe asthma in younger and adult patients who developed asthma early in their childhood life [18]. Considering family history of asthma, cases with positive family history of the disease showed a non-significantly increased level of tIgE compared to cases without family history of asthma. Therefore, the risk effect of family history might have not influenced IgE level and both groups of patients were within the positive range of IgE. Such findings may suggest that family history is IgE-independent risk factor for asthma. In a meta-analysis study, it has been demonstrated that family history of asthma and increased tIgE level were significantly associated with developing asthma [19].

This study also disclosed that median level of IgE showed some variations among asthma cases distributed according to the type of allergen that was suggested to provoke the disease. However, these differences were not significant. Sensitization to allergens is prominent in asthma development, and although associations between inhalant airborne allergens and asthma have been established for many years, they have recently been re-introduced as risk factors for asthma [20].

In conclusion, the present study re-enforce the significant role of IgE in patho-physiology of bronchial asthma in Iraqi patients. Its correlation with disease severity and allergen type was also suggested.

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أهمية مستوى IgE الكلي والخاص في الربو لدى المرضى العراقيين البالغين

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الملخص

تم إجراء الدراسة للمرضى والأصحاء خلال الفترة من كانون الثاني إلى حزيران 2019م على 104 مريضاً يعانون من مرض الربو (50 من الإناث و54 من الذكور) و111 من الأشخاص الأصحاء يتطابقون مع المرضى (54 من الإناث و57 من الذكور) لتقييم دور مستوى الكلوبولين المناعي E (IgE) في الفسيولوجيا المرضية للربو. وكان متوسط العمر \pm الانحراف المعياري للمرضى الربو 37.9 ± 12.1 سنة، بينما كان أقل بين عينات السيطرة (35.9 ± 10.5 سنة)، لكن بدون فرق معنوي. تم توزيع الذكور والإناث متساوي بين عينات الربو (48.1 و51.9% على التوالي) وعينات السيطرة (48.6 و51.4% على التوالي). لوحظ أن معظم حالات الربو لديهم مرض خفيف (49%)، تليها الربو المعتدل والشديد (93.4 و11.5% على التوالي). كما لوحظ أن 51.9% من المرضى لديهم تاريخ عائلي من الربو. فيما يتعلق بنوع مسببات الحساسية، كانت 21.2% من حالات الربو إيجابية مصلية لأنواع مختلفة من مسببات الحساسية. كان المستوى المتوسط لـ IgE مرتفعاً بشكل ملحوظ في مصل حالات الربو (204.1 نانوغرام / مل) مقارنة بعينات السيطرة (163.3 نانوغرام / مل؛ $p < 0.001$).

المستوى موازي بشكل إيجابي لشدة المرض، وقد لوحظ أعلى مستوى في الحالات الشديدة (244.9 نانوغرام/ مل، بينما تم تسجيل أدنى مستوى في الحالات الخفيفة (189.7 نانوغرام/ مل)، وأظهر مستوى IgE بعض الاختلافات بين حالات الربو الموزعة وفقاً لنوع مسببات الحساسية، وهي الأعلى تم تسجيل المتوسط في الحالات المصلية (215.2 نانوغرام/ مل، في حين لوحظ أدنى مستوى في الحالات الإيجابية المصابة لوبر الحيوانات (189.3 نانوغرام/ مل).

وكشف تحليل الانحدار اللوجستي أن إجمالي مرضى الربو، وكذلك حالات الذكور والإناث كانت في خطر متزايد لتطوير نوبات الربو بسبب ارتفاع مستوى IgE في المصل. وأظهر تحليل ROC أن مستوى المرتفع لـ IgE في المصل احتل منطقة كبيرة تحت المنحنى (AUC)، والتي كانت 0.828 (قيمة $p = 0.001$). في الختام، أكدت الدراسة الحالية تعزيز دور IgE في الفسيولوجيا المرضية للربو القصبي وارتباطه بشدة المرض ونوع مسببات الحساسية لدى المرضى البالغين.