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Evaluation of Total Serum Immunoglobulin E Levels in Asthma Patients

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Abstract

Background and aim: Asthma is a chronic inflammatory airway disease mediated by immunologic mechanisms. The pathogenic role of immunoglobulin E (IgE) is always associated with the development of allergic diseases, especially asthma because it is highly responsive to the allergen. The present study aimed to estimate and compare total serum IgE levels in mild, moderate, and severe asthmatics and healthy controls (HC) and to obtain the relationship between serum IgE levels and asthma. **Materials and Methods:** For the present study, a total of 200 subjects that, include 100 asthma patients and 100 HC, were recruited from the south Indian population with prior consent. Spirometry with reversibility testing was done in all cases, and serum IgE levels were estimated using a sandwich enzyme-linked immunosorbent assay kit. **Result:** The total IgE levels were significantly high in asthmatics than in HC ($P < 0.001$). Total IgE levels in normal, mild, and moderate asthmatics were 121.51 IU/mL, 382.78 IU/mL, and 540 IU/mL, respectively, and no severe asthmatics were found in the study. Serum total IgE levels in allergic asthma patients were significantly higher than in smoking and family history of asthma patients. The receiver operating characteristic curve was constructed to compare the total IgE levels in asthma patients versus HC, and the area under the curve for IgE was 0.62 (95% CI: 0.541–0.700), indicating the test was satisfactory with a statistically significant at $P < 0.005$. **Conclusion:** Total IgE levels were found to increase as the severity of asthma increased. Total IgE is essential in asthma and may be used as a diagnostics marker.

Keywords: Asthma, Chronic respiratory disease, South Indian population, Total IgE

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Introduction

Asthma is a complex inflammatory disease mediated by immunologic mechanisms.[1] It is a common condition due to inflammation of the lower respiratory tract. All over the world, nearly 300 million individuals are affected by asthma, and it affects all age groups and is increasing in prevalence in many developing countries. Environmental factors like air pollution, tobacco smoke, urbanization, and inherent biological and genetic susceptibilities are leading causes of asthma.[2] It is characterized by episodic or persistent wheezing, dyspnea, and cough.[3] Asthma can be categorized as allergic or non-allergic. Allergic (extrinsic) asthma is an immunologically mediated asthma orchestrated by immunoglobulin E (IgE) — an antibody generated by the immune system in response to a normally harmless substance.[4] In contrast, non-allergic (intrinsic) asthma is not typically triggered by exposure to a substance and is not associated with IgE. IgE plays a critical role in a specific allergic response; however, it can sometimes be elevated for other reasons, such as inflammatory diseases.[5]

IgE is a type of antibody produced by plasma cells in lymph nodes draining the site of antigen entry or, locally, at the sites of allergic reactions, by plasma cells derived from germinal centers developing within the inflamed tissue.[6] IgE is pathogenic in allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, and food allergy.[7] The pathogenic role of IgE antibodies in triggering and maintaining allergic inflammation in response to allergens is due to the binding of multivalent allergens to allergen-specific IgE on sensitized effector cells. These interactions trigger effector cell activation, release potent inflammatory mediators, recruit inflammatory cells, antigen presentation, and produce allergen-specific antibody responses.[8] Hence, specific IgE antibodies can be essential in identifying the relevant allergen and providing a therapy guide.[9] Further, the elevated level of total serum IgE may demonstrate the allergic etiology of asthma in the subjects studied.[2,10]

In asthma patients, total serum IgE levels are higher than in non-asthmatics.[2,11] The normal range for total serum IgE levels is 1.5–144 kU/l (1 kU/l = 1 IU/ml). 7, and the upper limit for total serum IgE levels is 195.1 IU/ml high. However, other studies proposed a value of more than 300 IU/ml to consider it pathologic.[1]

Various studies have found an association between total serum IgE levels and the prevalence of asthma, independent of specific reactivity to common allergens or symptoms of allergy.[4,12,13] Very few studies on IgE related to asthma are available in south India. The present study was designed to estimate and compare total IgE levels in healthy controls (HC) and asthmatics subjects and to obtain the relationship between total IgE levels and the disease severity.

Materials and Methods

Subjects and Ethics

A total of 200 subjects were recruited for the present study, which includes 100 Asthma patients (Bhagwan Mahavir Medical Research Centre and Gandhi Hospital in Hyderabad, India) and 100

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HC f. All asthma patients met the standards of asthma diagnosis as established by the American thoracic society criteria based on the Spirometry/Pulmonary function test (PFT), IgE, and patients with a history of Current/recent past upper respiratory tract infections and the presence of autoimmune disorders are excluded from the study. In addition, HC with no personal or family history of asthma were selected as the control group. The ethics committee reviewed and approved the study design (Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Hyderabad, India). Written informed consent forms were obtained from all subjects before the study.

Sample Collection

A 5 ml venous blood sample was drawn from each study participant, aliquoted in serum clot activator tubes, centrifuged at 600 rpm for 10 min, separated, and stored at −20°C in cryovials until analysis.

Total Serum IgE Assay

Principle

The immobilization occurs during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-IgE antibody. On mixing a monoclonal biotinylated antibody and a serum containing the native antigen, reaction results between the native antigen and the antibody, forming an antibody-antigen complex. Antibody-antigen bound fraction is separated from the unbound antigen. Another antibody labeled with the enzyme is added, which forms an enzyme-labeled antibody-antigen-biotinylated-antibody complex. A substrate is added to produce color measurable with a spectrophotometer.

Procedure

Total serum IgE (IU/ml) was measured using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (Accubind, CA, USA) in asthma patients and HC (n = 300). All reagents and working standards were prepared according to the manufacturer's instructions. The absorbance was read using an ELISA reader (BIO-RAD) at 450 nm and 630 nm dual filters. All the samples were thawed only once and assayed in duplicate.

Calculation of results

A standard curve was constructed by plotting the mean absorbance obtained for each reference standard against its concentration in IU/ml on graph paper, with absorbance on the vertical (Y) axis and concentration on the horizontal (X) axis. The concentration of IgE in IU/ml was determined from a standard curve using the mean absorbance value of each sample.

Statistical Analysis

The differences in the distribution of clinical characteristics of two study groups (patients and HC) were evaluated using the Student's t-test (continuous variables) $\alpha\nu\delta$ χ^2-test (for categorical variables). Clinico-demographic characteristics were expressed in Mean ± Standard Deviation (SD). A *P*-value of logistic regression analysis with a stepwise approach was applied for variables such as age, gender, BMI, PFT, allergic, smoking, and IgE. The GraphPad Prism software (version 7.0) was used for the IgE analysis and one-way analysis of variance (ANOVA) using SPSS, and the model with the most significant area under the curve (AUC) was considered.

Results

Demographic Characteristics

One hundred asthma patients (44 males and 56 females) and 100 HC (60 males and 40 females) were recruited for the study. Table 1 demonstrates the demographic characteristics and biochemical profile of asthmatics and HC. Among the study group, the number of males is predominant in HCs, while females are in asthma. The mean age of asthma patients was 39.67 ± 14.88, and HC was 32.35 ± 12.27, respectively. The BMI was 22.87 ± 26.52 and 25.49 ± 2.50 among patients and HC with a statistical difference at $P < 0.05$. The mean PFT forced expiratory volume 1/forced vital capacity (FEV1/FVC Post) values were 95.74 ± 12.15 and 29 ± 10.93 in both study groups, with a $P < 0.05$. Significantly elevated serum IgE IU/L levels were observed in asthma patients 316.54 ± 183.01 compared to HC 243.84 ± 131.56. The mean of smoking in patients is significant at 0.20 ± 0.40 compared to HC is 0.04 ± 0.20. The mean of HC is <0.03 ± 0.17 compared to asthma patients with allergy, at 0.80 ± 0.40 with a significant difference at $P < 0.05$. The mean of patients with a family history of asthma is 0.50 ± 0.50, statistically significant compared to HC of 0.02 ± 0.14 with $P < 0.05$.

Total Serum IgE Levels

The mean total IgE levels were found to be significantly high at $P < 0.001$ in asthma patients (316.5 ± 18.30) compared to HC (243.8 ± 13.16) Figure 1. In this study, the asthmatics were categorized into three groups mild, moderate, and severe based on the Global initiative for asthma (GINA) guideline Table 2. The patients in each group had similar demographic characteristics. Their PFT were significantly different among the three groups. Figure 2 shows a significant difference in IgE mean in asthma patients with smoking (316 IU/mL), allergic disease (336 IU/mL),

Table 1: Demographic characteristics of asthma patients and healthy controls

Characteristics	*Asthma patient (n=100) Mean±SD*	*Healthy control subjects (n=100) Mean±SD*	*P-value*
Sex (Male/Female)	44/56	60/40	-
Age (years)	39.67±14.88	32.35±12.27	*P*=0.0002
BMI (kg/m^2)	22.87±26.52	25.49±2.50	*P*=0.327
PFT (FEV1/FVC Post)	95.74±12.15	90.29±10.93	*P*=0.0001
Smoking	0.20±0.40	0.04±0.20	*P*=0.0004
Allergy	0.80±0.40	0.03±0.17	*P*=0.0001
Family history of asthma	0.50±0.50	0.02±0.14	*P*=0.0001
Total serum IgE, IU/L	316.54±183.01	243.84±131.56	*P*=0.0015

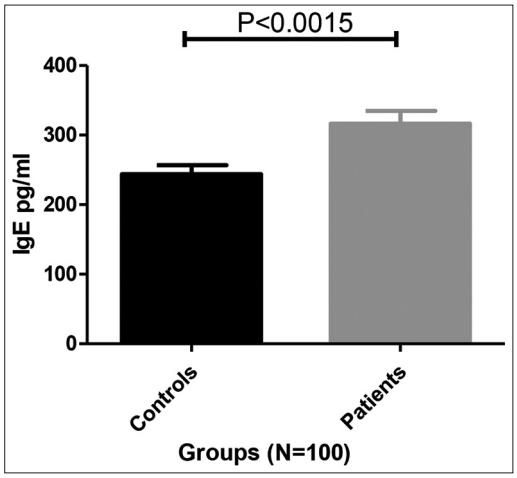

Figure 1: Total serum IgE levels in asthma patients versus healthy controls

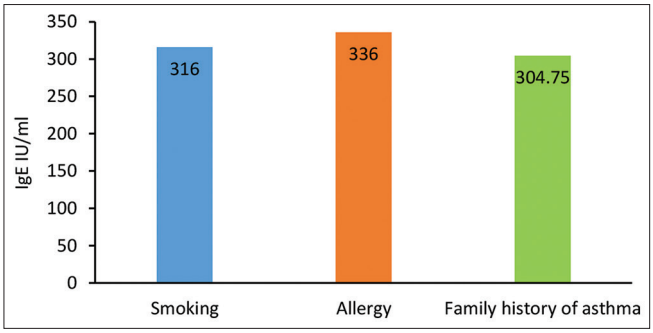

Figure 2: Total serum IgE in asthma patients with smoking, allergy, and family history. $P = 0.04$ (significant at $P < 0.05$)

and family history of asthma (304.75 IU/mL) at $P < 0.05$.

ANOVA

The comparisons between groups were carried out using variance analysis ANOVA. Our results of ANOVA illustrate a significant difference in the age, gender, FEV1/FEV post, smoking, allergy, family history of asthma, and IgE levels between the subjects at $P < 0.05$. ANOVA with *post hoc* test for comparisons using the Tukey HSD and Bonferroni test applied for all the markers confirmed that there was a significant difference in mean levels between the group's Table 3.

Receiver Operating Characteristic Curve (ROC) Curve Analysis

The ROC curves were constructed to compare the total IgE levels in asthma patients versus HC. The AUC for IgE was 0.62 (95% CI: 0.541–0.700), indicating the test was satisfactory with statistically significant at $P < 0.005$ Figure 3. The sensitivity and specificity were 70% and 54%, and the value cut was 192 for the serum levels. Regarding IgE, the subjects with IgE of <192 were considered high risk, while those above this threshold were at low-risk Table 4.

Table 2: Comparison of total serum IgE levels among mild, moderate, and severe asthma

Groups	*No. of subjects*	*Mean (IU/ml)*
Normal	41	121.51
Mild asthma	33	382.78
Moderate asthma	26	540.0
Severe asthma	–	–

Table 3: One-way ANOVA analysis for clinical parameters in asthma patients and HC

Variable	*Comparisons*	*Degrees of freedom*	*F statistic*	*P-value*
Age	Between groups	1	14.411	0.000
	Within groups	198	–	–
	Total	199	–	–
BMI	Between groups	1	3.156	0.077
	Within groups	198	–	–
	Total	199	–	–
Gender	Between groups	1	5.211	0.024
	Within groups	198	–	–
	Total	199	–	–
FEV1/FEV post	Between groups	1	10.532	0.001
	Within groups	198	–	–
	Total	199	–	–
Smoking	Between groups	1		
	Within groups	198	12.774	0.000
	Total	199	–	–
Allergy	Between groups	1		
	Within groups	198	310.402	0.000
	Total	199	–	–
Family history of asthma	Between groups	1		
	Within groups	198	84.605	0.000
	Total	199	–	–
IgE	Between groups	1		
	Within groups	198	10.403	0.001
	Total	199	–	–

Discussion

IgE plays a central role in the pathogenesis of allergic diseases, including asthma.[14] Increased serum IgE levels in asthma may be due to increases in IgE-dependent processes and cellular components of the immune system and events associated with IgE-dependent processes, which are crucial in asthma pathophysiology.[15] Higher IgE levels indicate some inherent susceptibility (atopy) and the presence of a disease process involving airway inflammation.[16-18]

The present study explored the total serum IgE levels in asthma patients and HC. IgE levels were significantly higher in the study group than in HC, and high IgE levels in patients might indicate inflammation in the disease. Concerning IgE levels in the present study, Al Obaidi *et al*. reported a similar result in asthma patients in Baghdad, Iraq.[19] Studies explained increased IgE levels in childhood asthma in the North Indian population, and the results are significantly associated with the disease.[16] Serum-free IgE level is a useful serologic marker for determining atopic status to differentiate between adults with asthma and HC without atopy. Woo *et al*. found significant associations between serum-free IgE levels and type 2 inflammation markers in the South Korean population. Patients with asthma with type 2 markers had higher free IgE levels and lower lung function.[20]

We found that the mean values of total IgE levels in normal, mild, and moderate were 121.51 IU/mL, 382.78 IU/mL, and

Table 4: Comparison of total serum IgE levels between patient and control (HC) groups

Values	*AUC*	*Std error*	*95%Confidence interval*	*P-value*	*Cut-off*	*Sensitivity*	*Specificity*
IgE	0.62	0.041	0.541-0.700	<0.005	192	70%	54%

AUC: Area under the curve, HC: Healthy controls, IgE: Immunoglobulin E

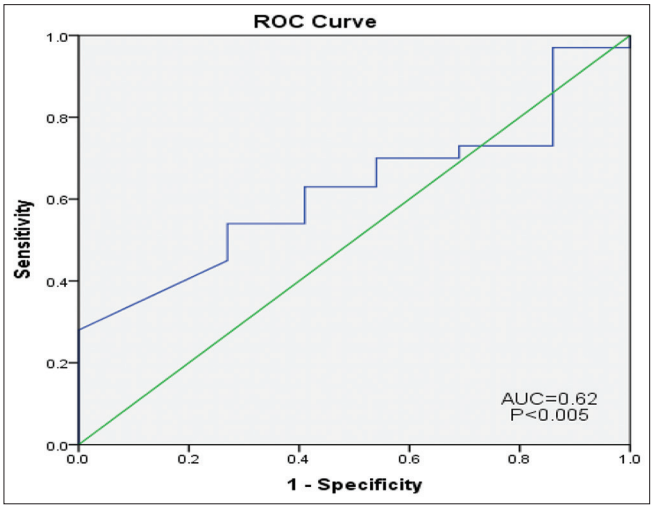

Figure 3: ROC curve

540 IU/mL, respectively. They were statistically significant when the levels were compared between these three groups ($P < 0.001$). Our study correlates with Sandeep *et al*.'s study, which investigated serum IgE levels in asthma patients and HC according to the GINA classification.[21] Studies reported that mean IgE levels in the North Indian population were significantly correlated with the disease severity.[16] The absence of severe asthmatics in the present study may be due to the management and control of asthma with predominant treatment via medications and lifestyle interventions of patients.[22]

Total serum IgE in asthma patients with smoking, allergy, and family history is statistically significant. The mean value of allergy is high at 336 IU/ml compared to smoking and a family history of asthma which explains the relationship of IgE with allergic diseases. This observation is consistent with a more severe degree of airway inflammation. Platts-Mills reported that "asthma is almost always associated with some type of IgE-related reaction and therefore has an allergic basis."[23,24] Sporik *et al*. observed that numerous epidemiologic studies have shown a highly significant relationship between asthma and sensitization to various allergens (as demonstrated by skin tests or specific IgE in the serum).[25-28] IgE is undoubtedly of central importance in both immediate hypersensitivity and the late-phase responses characteristic of allergy and asthma.[23,29,30] Exposure to tobacco smoking was an environmental factor that caused the triggered asthma and an increase in IgE levels. Our study found that patients exposed to smoking had high IgE levels of 316 IU/ml. Oryszczyn *et al*.[31] reported that the IgE level in smoker asthmatics was 3.5 times greater than in non-smoker asthmatics, which explains the relationship with smoking. Armentia *et al*. explain that Subjects exposed to cigarette smoke through either active or passive routes may increase airway responsiveness and affect IgE levels.[32-34] Family history of asthma patients with total IgE levels was low at 304.75IU/ml compared to allergy and smoking asthma patients. Abo-Shanab *et al*. study demonstrates that asthma is strongly related to a family history of allergy where IgE levels were found high in asthmatic patients suggesting its association with underlying symptoms.[35] Hameed *et al*. explains that the presence of an IgE-mediated allergic reaction in patients with asthma is often related to a family history of the disease.[2]

Conclusion

Our study explained that the serum IgE levels were higher in asthmatics compared to healthy subjects and increased progressively as asthma severity increased from mild to severe. It helps the way for a better understanding of the nature of the disease and its progression. Asthma patients' IgE levels were compared between the three groups of allergy, smoking, and family history, and the result is spastically significant. Asthmatics with allergies have high IgE levels compared to the other two groups. It explains that IgE may be involved early in the inflammatory cascade and can cause allergic asthma. ANOVA results explain that elevated IgE levels indicate a high possibility of asthma in the patients. IgE was considered a helpful biomarker based on the ROC curve analysis with significant sensitivity and specificity; hence may be used as a diagnostics marker. The information thus generated would help develop novel therapeutic strategies for treating an array of asthma with additional prospective studies.

Declaration Of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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