

# Relevance of Serum IgE Estimation in Allergic Bronchial Asthma with Special Reference to Food Allergy

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**SUMMARY** Studies suggest the importance of serum total and specific IgE in clinical evaluation of allergic manifestations. Such studies are lacking in Indian subcontinent, though a large population suffer from bronchial asthma. Here relevance of serum total and specific IgE was investigated in asthmatics with food sensitization. A total of 216 consecutive patients (mean age 31.9 years, S.D. 11.8) were screened by various diagnostic testing. Out of 216 patients, 172 were with elevated serum total IgE (201 to > 800 IU/ml). Rice elicited marked positive skin prick test reactions (SPT) in 24 (11%) asthma patients followed by black gram 22 (10%), lentil 21 (9.7%) and citrus fruits 20 (9.2%). Serum total IgE and specific IgE showed significant correlation,  $p = 0.005$  and  $p = 0.001$ , respectively, with positive skin tests. Blinded food challenges (DBPCFC) with rice and or black gram confirmed food sensitization in 28-37% of cases. In summary, serum total IgE of 265 IU/ml or more with marked positive SPT (4 mm or more) can serve as marker for atopy and food sensitization. Specific IgE, three times of normal controls correlates well with positive DBPCFC and offers evidence for the cases of food allergy.

Inflammation is the hallmark of bronchial asthma, which results in airway hyperresponsiveness and resistance to flow in the lower airways of lungs.<sup>1,2</sup> Various stimuli such as environmental allergens, psychic disturbances, infection, etc. are responsible for triggering inflammation and causing mild, moderate or severe airflow obstruction in asthmatics.<sup>3,4</sup> After discovery of IgE antibody,<sup>5</sup> attempts have been made to classify extrinsic asthma from intrinsic asthma.<sup>6</sup> Earlier studies suggest the importance of serum total IgE in the pathophysiology of asthma and the development of airflow obstruction.<sup>3,4,7</sup> Besides, a close association has been reported between the presence of elevated total IgE levels and symptomatic asthma.

Incidence of asthma has increased world over including Asian countries.<sup>8,9</sup> In India about 2-

8% population is estimated to suffer from bronchial asthma with or without rhinitis.<sup>8</sup> Like pollen and other allergens, foods are also known to cause severe systemic reactions and asthma in atopic individuals. But in India, very few attempts are made to study food sensitization and the allergic manifestations.<sup>10,11</sup> Currently, the methods used to diagnose food allergy include skin prick tests, measurement of food specific IgE, oral food challenges and double blinded placebo controlled food challenge. Determination of serum total IgE distinguishes atopic and/or non-atopic status of individuals.<sup>12,13</sup> Skin prick testing

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with allergen extracts is carried out to find out sensitization against offending allergens. Specific IgE estimation against respective allergen offers further evidence<sup>14,15</sup> to skin test results. However, all these methods of diagnosis, even double blinded placebo controlled food challenge may sometimes be misleading.<sup>16</sup> In view of the shortcomings in individual methods, the present study was undertaken to validate relevance of specific IgE estimations in diagnosing food allergy in asthmatics. Serum total IgE levels in these patients were also correlated with skin prick tests, oral food challenge and pulmonary obstruction.

## MATERIALS AND METHODS

### Subjects

Two hundred sixteen patients of bronchial asthma with or without rhinitis were included in the present study from referred cases to Out Patient Department of V.P. Chest Institute, Delhi, in the years 2003-2004. These patients aged 8-65 years comprised 115 (53.2%) males and 101 (46.7%) females. The patient's were with history of food allergy as recorded using a standard questionnaire.<sup>17</sup> The diagnosis of asthma was made based on history and clinical investigation following the criteria of American Thoracic Society 1991.<sup>18</sup> More than 15% reversibility of airway obstruction with 200 µg of salbutamol was taken as criteria for diagnosing asthma. The rhinitis<sup>19</sup> was assigned to patients having any two of the symptoms such as sneezing, rhinorrhea,

nasal blockade, post nasal drip, etc. Various steps involved in patient selection and testing in present study are described in Fig. 1.

The patients (asthmatics) from both sexes and non-smokers were included with history suggestive of atopy (inclusion criteria). Patients with other associated pulmonary diseases including tuberculosis, COPD and systemic diseases, *e.g.* diabetes, hypertension and heart diseases were excluded (exclusion criteria) from the study. Patients with mechanical causes of airway obstruction, steroid dependent and psychologically unstable cases were also excluded.

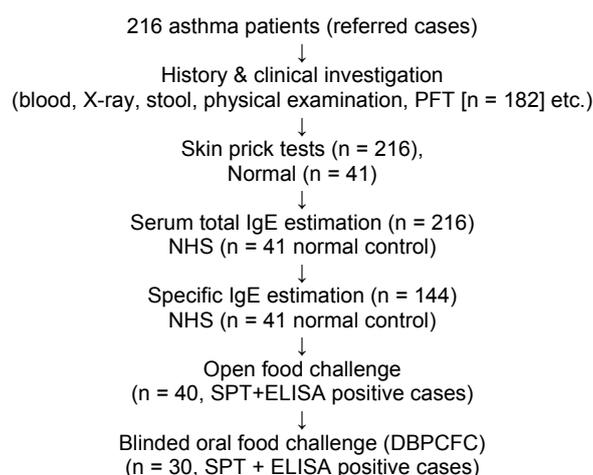
The written consent of each patient was obtained for participation in the study. The patients were persuaded to give blood for serum total and specific IgE estimation. The study protocol is approved by human ethics committee of the Institute.

### Pulmonary function test

Spirometric evaluation of patients was done as per American Thoracic Society guidelines<sup>18</sup> with Morgan Pulmolab equipment, England. The best of the three readings were taken for assessing their pulmonary function.

### Skin prick tests (SPT)

Skin tests were carried out in 216 patients with history suggestive of sensitization to food and



**Fig. 1** Flow chart describing the steps involved in patients' selection and testing for the present study.

common environmental allergens. The skin test reactions were graded after 20 minutes<sup>20</sup> in comparison to the wheal size of positive control, *i.e.* histamine diphosphate (5 mg/ml). Skin tests with mean diameter of wheal three mm and above were considered as marked positive reaction. For SPT, the allergen extracts (1:10 w/v, 50% glycerinated) were obtained from Antigen Laboratory, Institute of Genomics and Integrative Biology (CSIR), Delhi.

### Total IgE estimation

Serum total IgE estimation<sup>21</sup> was carried out for each patient and normal control by enzyme-linked immunosorbent assay (ELISA) using Pathozyne IgE kit (Omega Diagnostic Ltd, UK). Monoclonal anti-IgE was coated into wells as per manufacturer's instruction. After washing, patient's serum diluted 1:10 v/v with the zero buffer was incubated for 30 minutes at 25°C. Followed by washing, horse radish peroxidase conjugated anti-human IgE was added and incubated for 30 minutes. The color was developed by adding tetramethyl benzidine. The enzyme reaction was stopped with 100 µl of hydrochloric acid. Absorbance was read at 450 nm using Dynatech ELISA reader.

### Specific IgE estimation

Sera of patients showing marked positive skin reactions were evaluated by ELISA for specific IgE<sup>22</sup> against respective food extract. Briefly, 2 µg protein (extract) was coated (100 µl) per well in carbonate-bicarbonate buffer (pH 9.0) overnight at 4°C. After washing five times with phosphate buffered saline (PBS), unbound sites were blocked with 3% BSA. Following washing, wells were incubated with 1:10 v/v diluted patients' sera (100 µl) overnight at 4°C. The plate was washed again with PBS-Tween-20 and incubated with horse radish peroxidase conjugated anti-human IgE for 4 hours at 37°C. The color was developed using O-phenylenediamine. The reaction was stopped by adding 50 µl of sulphuric acid. Absorbance was read at 490 nm using an ELISA reader.

### Open food challenge (OFC)

In open food challenge test, the patients were given orally 20-60 g of suspected food(s) as per the history and kept under observation for 20 to 60 minutes. Spirometry evaluation was done before and af-

ter food challenge and fall in force vital capacity was recorded. OFCs were performed under medical supervision and patients were asked to stay in the clinic for at least 2 hours after the test. During the observation time blood pressure, pulse rate, forced expiratory volume in 1 second and peak expiratory flow were measured. Subjects showing a positive OFC were subsequently tested with DBPCFC.

### Double blinded placebo controlled food challenge (DBPCFC)

Double blinded placebo controlled food challenges (DBPCFCs) were performed as per the guidelines described earlier.<sup>23</sup> Briefly, the active food was masked to make it indistinguishable from the placebo and was administered in double blinded fashion, *i.e.* neither the patient nor the observer recording clinical manifestation knew about the content. The initial dose of food administered in DBPCFC was chosen based on the results of OFC. Approximately 20-30 g of the test food was sufficient to provoke allergic reactions in susceptible patients.

Spirometry evaluation, forced expiratory volume at one second (FEV<sub>1</sub>) was made prior and after food challenge. The airflow obstruction (PFT) was noted after food challenge test and obstruction of more than 15% in FEV<sub>1</sub> or 200 ml change was taken as significant value. The patients who developed symptoms such as breathlessness, cough, sneezing, rhinorrhea, vomiting and significant changes in spirometry were scored as DBPCFC positive. The patients were kept under observation for at least 2 hours after the food challenge test. In case of anaphylactic reaction as a result of provocation the patients were given epinephrine and avil and were nebulized with salbutamol and ipratropium bromide.

Same ingredients of placebo were used in both the active food and placebo. Placebo was selected from food materials which showed negative SPT, ELISA (Specific IgE) and oral challenge test in patients. The test recipe was usually prepared in the ratio of 3:1 (Placebo:test food).

### Statistical analysis

The statistical analysis was performed using SPSS (Statistical Package for Social Sciences) ver-

sion 10.5 software. The data was analyzed for all variables using the Chi-square test. The comparison was made between serum total IgE levels with pulmonary function, serum total IgE with skin prick test and specific IgE with skin prick test and oral food challenge test. The differences were considered to be statistically significant at  $p = 0.05$ .

## RESULTS

Out of 216 patients included in the present study, 54 were suffering with asthma alone, whereas 162 had associated rhinitis. Most of the patients were in the age group of 21-40 years with mean age 31.9 (SD 11.8). Serum total IgE was estimated in all cases ( $n = 216$ ) by ELISA. Age wise distribution of total IgE values (IU/ml) has been presented in Table 1. Maximum patients ( $n = 56$ ) with elevated IgE (201 to  $> 800$  IU/ml) were in 21-30 years age group followed by 31-40 years ( $n = 54$ ) and least ( $n = 12$ ) were in 51 years and above group. The serum total IgE data was analyzed in two groups of patients: (a) bronchial asthma alone and (b) asthma with associated rhinitis (Table 2). Altogether 61 patients showed

total IgE in the range of 201-400 IU/ml, 28 patients, IgE 401-600 IU/ml and 44 patients showed IgE values, 601-800 IU/ml. Thirty-nine patients demonstrated serum total IgE  $> 800$  IU/ml.

Out of the 216 cases, pulmonary function test could be performed in 182 asthma patients to record air flow obstruction. The patients were categorized as normal ( $> 80\%$  of the predicted FEV<sub>1</sub>), mild (60-80% of the predicted FEV<sub>1</sub>), moderate (40-60% of the predicted FEV<sub>1</sub>) and severe ( $< 40\%$  of the predicted FEV<sub>1</sub>) on the basis of airflow obstruction. Pulmonary obstruction (FEV<sub>1</sub>) and serum total IgE values were analyzed for all asthmatics (with or without rhinitis) and presented in Fig. 2. The total IgE values in normal healthy volunteers (NHS,  $n = 41$ ) ranged from 6-231 IU/ml. Mean  $\pm$  2SD was calculated and values  $> 265$  IU/ml IgE were taken to classify ELISA cut off for patients with significantly elevated IgE levels (Fig. 2). Among the patients with mild ( $n = 55$ ) to moderate ( $n = 40$ ) airflow obstruction, 40 and 27 patients, respectively demonstrated remarkably elevated IgE levels (265 IU/ml). Severe airflow obstruction was recorded in 19 cases

**Table 1** Serum total IgE values in different age groups of asthma patients

Age group (years)	Total IgE (IU/ml)					Total
	6-200	201-400	401-600	601-800	$> 800$	
08-20	09	08	02	10	10	39
21-30	17	16	11	16	13	73
31-40	07	20	09	14	11	61
41-50	07	11	03	03	03	27
51 & above	04	06	03	01	02	16

**Table 2** Distribution of serum total IgE in two groups of asthma patients

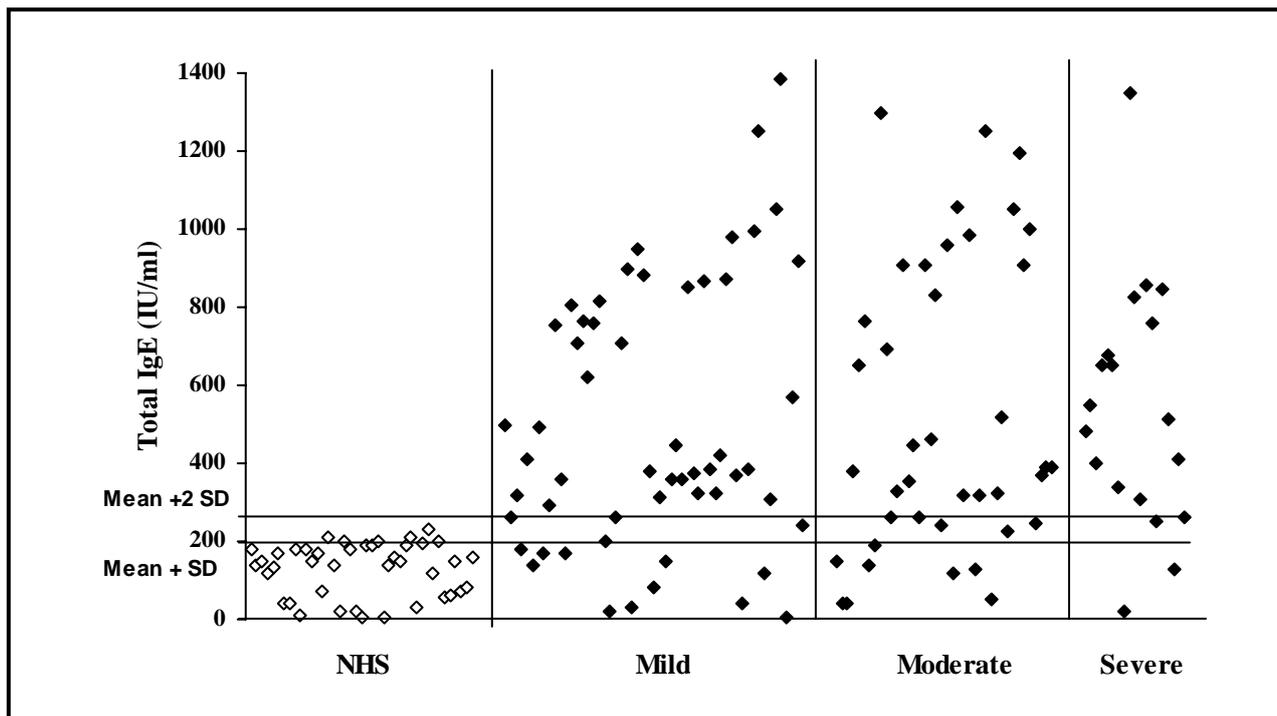
Total IgE (IU/ml)	Bronchial asthma No.	Asthma with rhinitis No.	Total No. (%)
6-200	11	33	44 (20.4)
201-400	14	47	61 (28.2)
401-600	11	17	28 (13.0)
601-800	12	32	44 (20.4)
$> 800$	06	33	39 (18.1)

and 15 of them demonstrated significantly elevated IgE levels. Comparative analysis of the data showed no statistically significant correlation ( $p = 0.815$ ) between serum total IgE levels and pulmonary obstruction (PFT).

SPT reactivity of these patients ( $n = 216$ ) was evaluated with different food extracts (Table 3). Rice extract elicited marked positive SPT in 24 (11%) patients followed by black gram 22 (10%), lentil 21 (9.7%) and citrus fruits 20 (9.2%). The food extracts such as pea, maize, French bean, Lima bean, fish and mustard exhibited marked positive skin reactivity in 5-6% patients. Many food extracts demonstrated marked positive skin reactions in 2-4% of the cases tested. Beside food extracts, these patients were skin tested with 9 dominant pollen allergen extracts. Some of the patients positive to foods also showed marked positive skin reactivity to pollen extracts. This may be due to co-sensitization or presence of cross reactive proteins in the extracts.

Specific IgE estimations were carried out in sera of patients against 9 major food allergens (Table 4). About 60-91% of SPT positive patients showed raised specific IgE levels. Specific IgE values three times of normal control ( $OD = 0.27$  or more) were designated as ELISA positive. Comparative analysis of marked positive SPT and specific IgE ( $n = 144$ ) showed significant correlation ( $p = 0.001$ ). Serum total IgE values ( $n = 216$ ) showed highly significant correlation ( $p = 0.005$ ) against marked positive skin tests.

Open food challenge (OFC) was carried out in 40 SPT and ELISA (specific IgE) positive asthma patients. The OFC was positive in 27 (67.5%) cases tested with rice, black gram, citrus fruits, banana, French bean and lentil. DBPCFCs conducted with rice ( $n = 16$ ) and black gram ( $n = 14$ ) in SPT positive cases, demonstrated positive challenges in 6 (37.5%) and 4 (28.5%) cases, respectively. The patients' suffered with breathlessness, cough, sneezing, rhinor-



**Fig. 2** Analysis of total IgE levels and airflow obstruction in bronchial asthma patients. Out of 182 asthma patients, 55 showed mild, 40 moderate and 19 severe airflow obstruction. The remaining 68 asthmatics showed normal airflow obstruction at the time of testing (data not included) mean + 2 SD of normal human sera (NHS,  $n = 41$ ) was calculated as given in parenthesis (ODs > 265 IU/ml).

rhea, vomiting, stomach upset etc. after the challenge test. Specific IgE and DBPCFCs with rice and black gram were correlated for 30 patients. The patients with specific IgE, three times of normal control or more ( $OD > 0.27$ ) demonstrated positive DBPCFC.

## DISCUSSION

Open food challenge (OFC) and/or double blinded placebo controlled food challenge (DBPCFC) are recommended to achieve definitive diagnosis of food allergy.<sup>23</sup> DBPCFCs are cumbersome, associated with risk and results of OFCs are not always reliable. In recent years, some studies have related the total and specific IgE levels in serum and respiratory symptoms in patients sensitized to food or inhalant allergens.<sup>11,24,25</sup> Choi *et al.*<sup>26</sup> observed that sensitivity of skin test was higher than specific IgE test, whereas the specificity of IgE test was better than skin test. Therefore, both the tests

should be used in a complimentary manner. Sampson and Ho<sup>27</sup> have established cut off points in the specific IgE serum levels for different foods that can predict clinical reactivity after ingestion in 90-95% of the group studied. In the present study, relevance of serum total IgE levels was evaluated in terms of airway responsiveness, *i.e.* pulmonary obstruction in allergic asthmatics. The data for serum total IgE and specific IgE against respective food allergen were collected, analyzed and correlated for clinical use.

Previously serum total IgE levels were shown to be related with age, genetic predisposition, race, immune status and some disease processes.<sup>28</sup> The upper limit for IgE in normal adults has been recorded in the range of 40.9 IU/ml to 200 IU/ml in different studies.<sup>7,29</sup> Lindeberg and Arroyave<sup>30</sup> have defined 100 IU/ml as the cutoff to differentiate allergic and non-allergic subjects. For children less than

**Table 3** Skin prick tests (SPT) with food allergen extracts on patients of asthma (n = 216)

Sample number	Allergen extracts	2+ No.	3+ No.	4+ No.	Total	
					No.	%
1	Rice	23	1	-	24	11.1
2	Black gram	19	3	-	22	10.1
3	Lentil	21	-	-	21	9.7
4	Citrus fruits*	15	4	1	20	9.2
5	Pea	10	3	-	13	6.0
6	Maize	11	2	-	13	6.0
7	Lima bean	9	3	-	12	5.5
8	French bean	11	1	-	12	5.5
9	Fish	9	2	-	11	5.0
10	Mustard	10	1	-	11	5.0
11	Peanut	8	1	-	09	4.1
12	Banana	9	-	-	09	4.1
13	Soybean	5	1	1	07	3.2
14	Coconut	6	1	-	07	3.2
15	Chicken	4	3	-	07	3.2
16	Radish	5	1	-	06	2.7
17	Almond	5	-	-	05	2.3
18	Carrot	5	-	-	05	2.3
19	Cashew nut	5	-	-	05	2.3
20	Wheat	3	1	-	04	1.8

Total food extracts tested (n = 38); food extracts (n = 15) not included here showed SPT +ve reactions in < 1% cases; 2+ to 4+ = markedly positive skin reactions.

\*Citrus fruits include orange, grapes, musambi and lemon.

**Table 4** Specific IgE levels against food allergen extracts in skin prick test (SPT) positive asthma patients

Sample number	Allergen extracts	SPT +ve	Specific IgE positive*	
			No.	%
1	Rice	24	16	66.6
2	Black gram	22	16	72.7
3	Lentil	21	16	76.1
4	Citrus fruits	20	12	60.0
5	Pea	13	09	69.2
6	French bean	12	11	91.6
7	Lima bean	12	08	66.6
8	Mustard	11	07	63.6
9	Banana	09	07	77.7

\* ELISA positive = ODs > 0.27 (three times of normal control), Normal control = 0.09.

one year of age, the cut off value of 10 IU/ml provided 100% specificity and sensitivity. In the present study, 265 IU/ml serum total IgE (mean  $\pm$  2SD of NHS) was taken as cutoff for ELISA positive. Based on this, 82 (45.1%) asthmatics showed mild, moderate or severely impaired lung function (Fig. 2). The patients with significantly elevated IgE levels (201 to > 800 IU/ml) were in 21- 40 years age group. But no statistically significant correlation was observed between age and total IgE levels in our study ( $p = 0.12$ ). Burrows *et al.*<sup>12</sup> reported similar findings about serum total IgE and rate of disease in different age group patients. Shadick and co-workers<sup>31</sup> observed association between serum total IgE concentrations at the early life with lower level of pulmonary function. But it is not predictive of accelerated rate in decline of FEV1 among middle aged and older man. Taylor and coworkers<sup>32</sup> observed in a 7.5 years follow up study, that decline in FEV1 was unrelated to total serum IgE levels. This is in agreement to our findings since no statistically significant correlation ( $p = 0.815$ ) was observed between total IgE levels and pulmonary obstruction in allergic asthmatics.

Earlier studies suggest that quantitation of food specific IgE is a useful test for diagnosing allergy to milk, egg, peanut and fish.<sup>11,33</sup> This could eliminate the need of double blinded placebo controlled challenges in a significant number of subjects. Garcia-Ara *et al.*<sup>34</sup> also stated that, milk allergic

cases with positive SPT and 2.5 kU/l specific IgE or greater should not be subjected to oral food challenges. In the present study, many asthmatics showed food sensitization as evaluated by history and skin prick tests (SPT). Specific IgE estimation with 9 common food allergen extracts showed significantly raised levels in SPT positive cases. The data for marked positive skin tests and specific IgE showed statistically significant ( $p = 0.012$ ) correlation. Among 40 SPT and ELISA positive (specific IgE 3 times of control) cases, 27 (67.5%) patients gave OFC positive test. However, no statistically significant correlation ( $p = 0.608$ ) was observed between higher specific IgE and open food challenge positivity. DBPCFC carried out with rice and black gram showed positive challenges in 37.5% to 28.5% cases tested. The patients showing DBPCFC positive were with marked positive skin tests (4 mm or more wheal) and significantly elevated specific IgE (OD > 0.27, *i.e.* three times or more of normal control) against respective food.

In conclusion, elevated serum total IgE levels (> 265 IU/ml) indicate towards atopic status of patients, but it did not correlate with pulmonary obstruction in allergic asthmatics. Specific IgE three times of control and skin prick tests 4 mm wheal or more offer substantial evidence for food hypersensitivity in asthmatics. Based on this, the patients can be advised to undertake avoidance measures against the offending food allergen.

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