

A Descriptive Study on Serum IgE and Absolute Eosinophil Count in Children with Bronchial Asthma Attending the Paediatric Department of a Medical College in Central Kerala

Sajna Gamaly Leela¹, Menon Narayanankutty Sunilkumar², Ramaraj Subbiah³

^{1, 2, 3} Department of Paediatrics, Amala Institute of Medical Sciences, Thrissur, Kerala, India.

ABSTRACT

BACKGROUND

Bronchial asthma is a major public health concern especially in the paediatric population. An elevated immunoglobulin E (IgE) level is considered as an objective marker of allergy. Because IgE is a mediator of allergic response, quantitative measurement of IgE together with other clinical markers, can provide useful information for the differentiation between atopic and non-atopic diseases. In this study, we wanted to determine serum IgE and absolute eosinophil count (AEC) levels and correlate both levels with severity of asthma in children in the age group of 2 - 15 years admitted in the paediatric department.

METHODS

This is a descriptive cross-sectional study. After taking the informed consent from the parent or guardian, relevant information on asthmatic symptoms and severity, other associated manifestations, precipitating factors, and family history of asthma were recorded in a pre-designed proforma. A total of 65 children (age group 2 – 15 years old) with bronchial asthma were studied. Investigations such as hemogram and serum IgE levels were determined. Asthma severity was assessed according to global initiative for asthma (GINA) guidelines.

RESULTS

Among 65 children, 69.2 % were boys. Receiver operating characteristic curve (ROC) analysis showed that total IgE cut-off concentration of 168 IU/ml, distinguishes children with intermittent asthma from those with mild persistent disease. IgE cut-off concentration of 989 IU/ml distinguishes mild persistent from those with moderate persistent asthma. Both showed adequate or good diagnostic efficacy. Cut off value of 168 IU/ml may prove useful in practice, indicating that 75 % of children in intermittent group will have serum concentration of total IgE < 168 IU/ml. No association was observed between AEC and increasing severity of asthma. Only atopic dermatitis with asthma showed increasing trend of IgE levels.

CONCLUSIONS

Asthmatic children with higher asthma severity have a higher serum total IgE concentration (168 IU/ml and 989 IU/ml are the cut off values for differentiating intermittent from mild persistent and mild persistent from moderate persistent).

KEYWORDS

Serum IgE, Absolute Eosinophil Count, Childhood Asthma, Asthma Severity

Corresponding Author:

*Dr. Menon Narayanankutty Sunilkumar,
Professor,
Department of Paediatrics,
Amala Institute of Medical Sciences,
Amala Nagar, Thrissur - 680555,
Kerala, India.
E-mail: sunilsree99@gmail.com*

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BACKGROUND

Asthma is a chronic inflammatory condition of the lung resulting in episodic airflow obstruction and this chronic inflammation heightens the twitchiness of the airways—airways hyperresponsiveness—to provocative exposures.¹ Recurrent wheezing episodes in early childhood are associated with common respiratory viruses, especially common cold rhinoviruses, and also respiratory syncytial virus, parainfluenza & influenza virus, adeno, and human metapneumonic virus. This implies that host features affecting inflammation, immunologic defense, and the extent of airways injury from ubiquitous viral pathogens underlie susceptibility to recurrent wheezing in early childhood.²

Home allergen exposure in sensitized children can initiate airways inflammation and hypersensitivity to other irritant exposures, and are strongly linked to disease severity and persistence.^{2,3} Consequently, eliminating the offending allergen can lead to resolution of asthma symptoms and can sometimes cure asthma. There are an array of allergens seen in nature which can produce symptoms of childhood asthma. Environmental tobacco smoke and common air pollutants can aggravate airways inflammation and increase asthma severity. Cold, dry air hyperventilation from physical play or exercise, and strong odours can trigger bronchoconstriction.¹⁻⁴

The clinical picture of asthma, i.e., the disease severity, results from a series of complex pathophysiological processes determined by numerous known and unknown factors from genetic to environmental. Immunoglobulin E (IgE) is a protein that normally accounts for less than 0.001 % of total serum immunoglobulin. Presence of allergen specific IgE on the cell surfaces of antigen presenting cells is a unique feature of atopy. Cross linking of receptor-bound IgE molecules by allergen initiates a complex intracellular signalling cascade followed by the release of various mediators of allergic inflammation from mast cells and basophils.^{1,5} Allergic diseases are characterized by peripheral blood and tissue eosinophilia. Eosinophils participate in both innate and adaptive immune responses and, like mast cells contain dense intracellular granules that are sources of inflammatory proteins.^{1,5,6}

Elevated IgE is considered as one of the markers of allergy and has been associated with many respiratory disorders. Since IgE is a mediator of allergic response, quantitative measurement of IgE and other clinical markers, can provide useful information to differentiate between atopic and non-atopic diseases. In the absence of parasitic infections, raised IgE and absolute eosinophil count can be considered as a hallmark of atopic sensitization often associated with airway obstruction.^{5,6,7,8}

Identification of disease severity is a precondition for appropriate therapeutic approach in asthma patients. Determination of serum concentration of total and specific IgE antibodies plays a major role in the diagnosis of childhood asthma. Serum concentration of IgE depends on the genetic predisposition to an increased IgE synthesis and exposure to a broad array of environmental allergens. IgE levels vary with the degree of immune stimulation, i.e., with

the frequency, dose and route of allergen exposure, respectively.⁶⁻⁸

Based on these findings, the current study was intended to correlate the levels of serum IgE and absolute eosinophil count in children with different grades of asthma in the age group of 2 - 15 years admitted in the paediatric department. Also, the study aims to correlate the levels of IgE & absolute eosinophil count in children in various categories: (a) children with only wheezing episodes, (b) those with wheeze and those with history of associated urticaria, allergic rhinitis, food allergy and (c) also compare both levels in children using inhaled steroids and those who are not using it. We wanted to determine serum IgE and absolute eosinophil count levels and correlate both levels with severity of asthma in children in the age group of 2 - 15 years admitted in the paediatric department.

METHODS

Children attending the paediatric department of Amala Institute of Medical Sciences, Thrissur a tertiary level medical college hospital, in Central Kerala were the study population. It was a descriptive cross-sectional study. After taking the informed consent from the parent or guardian, relevant information on asthmatic symptoms and severity, other associated manifestations, precipitating factors, and family history of asthma was recorded in a pre-designed proforma. A total of 65 children (age group 2 - 15 years old) with bronchial asthma were studied. The study was conducted from January 2017 to August 2018. The sample size was calculated based on literature search of past studies on the topic. Study conducted by Children's Hospital Sebrnjak, Zagreb, Croatia showed that cut off value of IgE of 156.3 kIU/L has a sensitivity of 42.1 % in interpreting mild persistent asthma.² Paramesh et al. demonstrated that asthma prevalence was significantly higher in urban children when compared to their rural counterpart. (16.6 %, 5.7 %).³ Sample size was calculated using the formula $Z^2 \times \{SN(1-SN)\} / W^2$; $1.96^2 \times 0.42 \times (1 - 0.42) / (0.05)^2$, where sensitivity = 42 %, z, confidence interval, 95 % = 1.96 W = 0.05, prevalence of asthma = 5.7. The calculated sample size was 65. After taking the informed consent from the parent or guardian, relevant information on asthmatic symptoms and severity, other associated manifestations, precipitating factors, and family history of asthma was recorded in a pre-designed proforma. Asthma was defined as paroxysms of dyspnoea, cough and wheeze of varying severity which resolves spontaneously or with therapy. Severity of asthma was categorized as intermittent, mild, moderate, or severe persistent based on symptoms and as per standard guidelines.

A hemogram and serum IgE levels were determined. Complete blood count (CBC) was obtained using auto-analyser; absolute eosinophil count was manually calculated. Serum IgE levels were measured using COBAS 6001 immunoassay analyser, an electro chemiluminescence immuno assay (ECLIA). It is a solid phase enzyme linked immunosorbent assay based on the sandwich technique. This instrument is calibrated to provide quantitative values

for serum IgE up to 1000 IU/ml. Values higher than 1000 IU/ml were expressed as > 1000 IU/ml, hence for the purpose of analysis this value is taken as 1000. An absolute total serum IgE value above 200 IU/ml was considered as elevated. An AEC of > 400 cells/mm³ was taken as suggestive of eosinophilia. Further, significant eosinophilia was graded based on AEC, Mild: 400 – 1500 cells/mm³, Moderate: 1500-5000 cells/mm³, Severe - >5000 cells/mm³.

Inclusion Criteria

The inclusion criteria included: 2 - 15 years old children admitted with wheeze, those using inhaled steroids for > 1 month, children with cough variant asthma and children who were given albendazole in last 6 months.

Exclusion Criteria

The exclusion criteria included those children on immuno suppression, children with parasitic infestations, tuberculosis, pneumonia, malnutrition, malignancy, congenital heart disease, cardiac failure, foreign body aspirations, cystic fibrosis and with bronchiectasis.

Statistical Analysis

The data obtained is entered in Microsoft Excel sheet and analysed using Statistical Package for Social Sciences (SPSS) version - 23. Results were expressed in percentage and proportions. Appropriate statistical technique like sensitivity, specificity, and Man-Whitney U-test, Kruskal-Wallis test, Spearman correlation test and ROC analysis were used for statistical analysis. P value < 0.05 was considered significant in our study. SPSS (Statistical Package for the Social Sciences), also known as IBM SPSS Statistics, is a software package used for the analysis of statistical data.

RESULTS

Out of the 65 children who had consented to be part of the study, there were a total of 45 (69.2 %) male and 20 (30.8 %) female children. The mean age of the study population was 5.97 years. 46 cases had family history of asthma (70.8 %) and 19 did not have (29.2 %). The Table 1 denotes the common triggers in the study population and the absence of the triggers were also noted.

The common triggers were identified, and details were noted. The common triggers were dust exposure, seasonal variation, exposure to smoke and strong smell, pollen, food allergy, pet exposure. Other points noted were exercise induced symptoms and wheezing following an acute upper respiratory infection.

The associated manifestations such as urticaria, atopic dermatitis and allergic rhinitis were noted. The clinical findings such as urticaria was present in 8 children (12.3 %), atopic dermatitis in 5 children (7.7 %) and allergic rhinitis in 2 children (3.1 %). So, in this study population, the children not having urticaria were 57 (87.7 %), atopic dermatitis was absent in 60 (92.3 %) and allergic rhinitis was absent in 63

children (96.9 %). According to the severity of asthma based on standard guidelines, the following were noted in the study population. 20 children had mild intermittent asthma (30.8 %), 23 had mild persistent asthma (23 %), 19 children had moderate persistent asthma (29.2 %) and remaining 3 were in the severe persistent group (4.6 %).

	Present		Absent	
	No. of Cases	Percentage	No. of Cases	Percentage
Seasonal	36	55.4	29	44.6
Dust	45	69.2	20	30.8
AURI	44	67.7	21	32.3
Food	4	6.2	61	93.8
Physical activity	17	26.2	48	73.8
Smoke	8	12.3	57	87.7
Pet exposure	0	0	65	100
Strong smell	17	26.2	48	73.8
Pollen	2	3.1	63	96.9

Table 1. Common Triggers in the Study Population

	Serum IgE	AEC
Area under the curve (AUC)	0.952	0.565
P value	0.0001	0.465
Cut off value	168	43.32
Sensitivity	95 %	60 %
Specificity	75 %	40 %

Table 2. ROC Analysis Intermittent to Mild Persistent Asthma

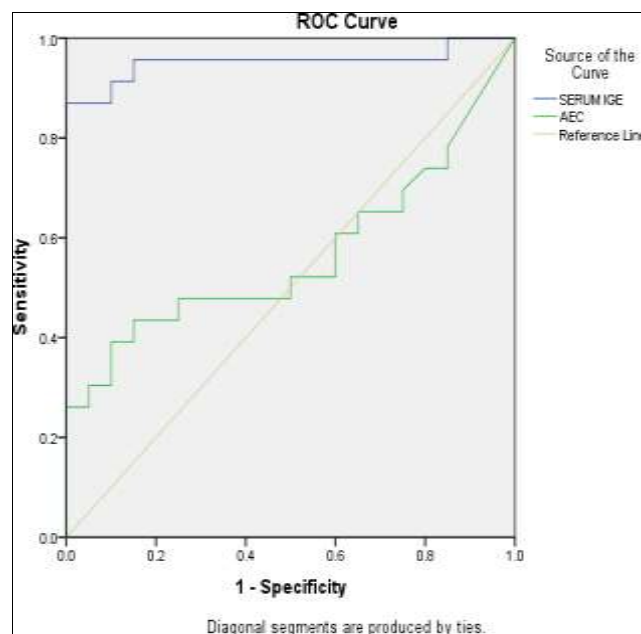


Figure 1. ROC Curve Analysis of Correlation of Serum IgE, AEC with Asthma Severity

The correlation of total IgE with asthma severity was expressed by use of ROC curve analysis. Due to small number of study children with severe persistent asthma, ROC analysis could not be performed for this patient group. The total IgE cut-off concentration of 168 IU/ml distinguish children with intermittent asthma from those with mild persistent disease and showed adequate or good (Intermittent: mild persistent AUC = 0.952) diagnostic efficacy (P < 0.0001). Cut off value of 168 IU/ml may prove useful in practice, indicating that in intermittent group 75 % of children have serum concentration of total IgE < 168 IU/ml, while 95 % of children in the mild persistent group had total IgE >168 IU/ml. AEC showed no statistically significant correlation (P - 0.465). Table 2 and Figure 1

explains the ROC analysis of intermittent to mild persistent asthma.

The total IgE cut-off concentration of 989 IU/ml, distinguish children with mild persistent from those with moderate persistent asthma, and showed adequate or good (mild persistent: moderate persistent AUC = 0.882) diagnostic efficacy (P < 0.0001). Cut off value of 989 IU/ml may prove useful in practice, indicating that in the mild persistent group 87 % of children have serum concentration of total IgE < 989 IU, while 89 % children with moderate persistent asthma will have total IgE > 989 IU/ml. AEC showed no statistically significant correlation (P - 0.206).

IgE levels showed statistically significant (P - 0.028) increasing trend in children with asthma and atopic dermatitis. AEC shows no significant change. Table 3 explains the IgE & AEC levels in children with asthma and asthma with atopic dermatitis

	Atopic Dermatitis	Number	Mean	Standard Deviation	P Value (Mann Whitney Test)
Serum IgE	Absent	60	529.14	378.29	0.028
	Present	5	934.86	145.65	
AEC	Absent	60	238.66	297.31	0.058
	Present	5	542.62	430.81	

Table 3. IgE & AEC Levels in Children with Asthma and Asthma with Atopic Dermatitis

In the whole of the study, we had only 3 children (4.6 %) who were on inhalers. 1 case in mild persistent group and 2 in moderate persistent group, that is not enough to find out the effect of inhalers on IgE levels and AEC, even though Mann Whitney test showed no positive correlation (IgE - P < 0.145, AEC - P < 0.521).

Table 4 explains the mean IgE and AEC with severity of asthma. The increasing trend of mean IgE levels with asthma showed a statistically significant trend (P = 0.001 - Kruskal Wallis test - since not following normal distribution), but increasing trend of AEC is not statistically significant, not following normal distribution, values changing widely (P = 0.151). Mean IgE levels of severe persistent group is 1000 because the COBAS 6001 immunoassay analyser is calibrated to provide quantitative values for serum IgE up to 1000 IU/ml. Values higher than 1000 IU/ml were expressed as > 1000 IU/ml, hence for the purpose of analysis this value is taken as 1000.

Severity of Asthma	Serum IgE	P Value (Kruskal Wallis test)	AEC	P Value (Kruskal Wallis Test)
Intermittent	Mean	137.90	125.63	0.001
	Standard deviation	69.58	118.35	
Mild Persistent	Mean	548.26	297.32	
	Standard deviation	247.62	403.56	
Moderate persistent	Mean	950.24	337.39	0.151
	Standard deviation	211.65	321.58	
Severe Persistent	Mean	1000.00	423.87	
	Standard deviation	.000	207.86	

Table 4. Mean IgE and AEC with Severity of Asthma

Table 5 explains the changes of IgE levels with changes of AEC. AEC showing increasing trend with serum concentration of IgE (P - 0.011).

Variable	Spearman's RHO Correlation Coefficient	P Value
Serum IgE	0.312	0.011

Table 5 Changes of IgE Levels with Changes of AEC

No association was observed between AEC and increasing severity of asthma. IgE level increases with AEC in asthmatic children (P = 0.011). Among children with wheeze and associated urticaria, atopic dermatitis and allergic rhinitis, only atopic dermatitis with asthma showed increasing trend of IgE levels (P = 0.028).

The limitations of this study were that there were only 3 children using inhalers. 1 child in the mild persistent group and 2 children in the moderate persistent severe group. This data was not enough to find out the effect of inhalers on IgE levels and AEC, even though Mann Whitney test showed no positive correlation (IgE - P < 0.145, AEC - P < 0.521).

DISCUSSION

Asthma is a non-curable but preventable disease, responsible for number of morbidities worldwide. The burden of morbidity is higher in developed countries and increasing in developing countries.⁹

The prevalence rate of asthma is higher among children living in houses using solid fuels (firewood - 80 %, kerosene - 78 %). One third of the cases can be eliminated by minimizing the use of any solid fuels. Around 17 % of all asthma cases in paediatric population could be attributed to underweight.⁹ A study done in Bangalore by Paramesh et al. demonstrated up to three times increase in the prevalence of asthma over a period of two decades and 80 % of all asthmatic patients reported disease onset prior to 6 years of age. However, of all young children who experience recurrent wheezing, only a minority goes on to have persistent asthma in later childhood.³

The study group of 65 asthmatic children (between age group 2 - 15 years of age) showed male predominance. Mean age at presentation in our study was 5.97 + 2.93 years. Positive family history of atopic diseases was recorded in 46 children (70.8 %). In most patients, initial symptoms occurred between 1 and 3 years of age. Kornelija et al. studied 157 asthmatic children which also showed a male predominance of 68.25 %² and positive family history in 49.7 % cases and initial symptoms occurred between 3 and 4 years of age.^{2,10}

Prevalence of various triggering factor noted, dust is the most commonly encountered triggering factor (69.2 %), followed by history of preceding acute upper respiratory tract infection (67.7 %) and seasonal variation (55.4 %). Table 1 explains these facts. Changes in housing allowing proliferation of house dust mite, the effects of indoor and outdoor pollutants, prematurity, low birth weight, dietary

changes, and passive cigarette smoke exposure may all account for the increased prevalence.¹¹

Atopy is defined as a personal and or familial propensity to produce IgE antibodies and sensitization in response to environmental triggers. Underlying atopy has been considered to be critical in linking atopic dermatitis, allergic rhinitis, and asthma. Literature review highlights that the risk of developing all atopic diseases is complex and strongly influenced by both genetic and environmental factors.¹² Children may have very specific triggers, for example, certain food or particular household cleaners. Or even stress may exacerbate asthma symptoms.¹⁰⁻¹² Rietveld et al.¹³ reported inducing breathlessness in adolescent children with asthma by getting them to play a frustrating game with a cash reward.

A total of 23 subjects (35.4 %) had mild persistent asthma and only 3 had severe persistent asthma (4.6 %). Study conducted by Anupama et al.¹⁴ showed 28 % children in mild persistent group, 33 % children in moderate persistent group and 39 % children in severe persistent group¹⁴ and in other study as well.¹

Role of differential chemical mediators are now recognized to be involved in asthma and mediate the complex inflammatory response in the airway.⁴ Large number of eosinophils were present in the airways of most individuals, but not all, persons with asthma.⁷ The increased number of eosinophils present in the airways, releases basic proteins which may damage the airway epithelial cells. They also had a role in airway remodelling and release of growth factors.⁸

Chemokines were important for the recruitment of inflammatory cells to the airways and are mainly located in airway epithelial cells.^{15,16} Eotaxin is selective for eosinophil, whereas macrophage-derived chemokines (MDS) and thymus activation-regulated chemokines (TARC) recruit Th2 cells. Cysteinyl leukotriene is a potent proinflammatory mediator and bronchoconstrictor, mainly derived from eosinophils and mast cells. These are the only mediators whose inhibition is associated with an improvement in asthma symptoms and lung function. Cytokines co-ordinate the inflammatory response and determine the severity of asthma. Interleukin IL-13, is important for IgE formation. Prostaglandin D2 is a bronchoconstrictor derived from mast cells.^{15,16,17}

The studies by Kornelija Kovac et al.¹ and Anupama et al. A⁹ showed significant increase in IgE levels in all the three groups of asthmatic patients when compared with normal control subjects ($P < 0.001$). The serum IgE level significantly increased with the severity of asthma predicted by pulmonary function tests ($P < 0.001$), i.e. the severe bronchial asthma displayed higher serum IgE than mild or moderate types. Thus, the serum IgE level was proportionately higher in patients with more severe airflow obstruction.¹⁴

The increased risk for males in childhood is probably related to narrower airways, increased airway tone, and possibly higher IgE in boys,¹⁴ which predispose them to enhanced airflow limitation in response to a variety of stimulus. Childhood asthma was reported to be more prevalent in boys than in girls in another study.¹⁷ This

difference disappears after the age of 10 years when airway diameter/length ratio is the same in both sexes, because of changes in thoracic size that occurs in puberty in males but not in females.¹⁸

The knowledge of the mechanisms of allergy is vital to understand these phenomena. The molecular mechanisms underlying immune system activation for allergen-induced asthma include stimulation of CD4 + Th2 (Type 2 helper cells) immune response and the subsequent production of IgE antibody. In atopic individuals, the IgE receptors, send unusually strong signals when cross-linked, resulting in secretion of abnormally high levels of IL-4 from mast cells, which further results in overproduction of IgE antibodies.^{15,19} Serum IgG and IgA levels increase along with IgE, whereas serum IgM levels decrease in bronchial asthma.^{14,15,17}

In the Jagadeeshwar et al.²⁰ study total IgE level was estimated by enzyme linked immuno sorbent assay (ELISA) and chemiluminescence method. Peripheral eosinophil (%) and absolute eosinophil count was estimated by PENTRA - 120 cell count. Peripheral eosinophil count was found to be highest in cases of sneeze and wheeze. A significant relationship exists between serum IgE levels and eosinophilia in populations presumed to be free of parasites where IgE levels presumably provide a better clue to atopy than do skin tests.²⁰ Esengul Keles et al.²¹ postulated that serum eosinophil cationic protein and total IgE predict the persistence of wheezing in young children. Ravindra Sonawano et al. in their study on children with allergic rhinitis with or without bronchial asthma, demonstrated the positive relation between nasal and peripheral smear eosinophil count.²²

Sudha S Deo et al. demonstrated the relationship of total IgE, specific IgE, skin test reactivity and eosinophils in a subset of patients with allergy.²³ Gupta et al. in their study in patients with bronchial asthma found that the serum IgE was elevated with hypereosinophilia.²⁴

Sandeep et al. in their study found that on an average, the levels of serum IgE increased as the severity of asthma increase.²⁵

Cissy B. Kartasamita et al. in their study found that, the overall median value of IgE was 436 IU/ml; almost 94 % of the children showed an IgE value of more than 100 IU/ml, and 29 % showed a value of more than 1000 IU/ml in bronchial asthma. IgE values and eosinophilia are markedly increased in these children under 5 years.²⁶

In the whole of the study, we got only 3 children who were on inhalers. 1 case in mild persistent group and 2 in moderate persistent, that is not enough to find out the effect of inhalers on IgE levels and AEC, even though Mann Whitney test showed no positive correlation (IgE - $P < 0.145$, AEC - $P < 0.521$).

The mean IgE and AEC concentration was lowest in children with intermittent asthma (137.9 + 69.5 IU/ml, 125.6 + 118.3 cells/mm³), higher in children with mild persistent asthma (548.26 + 247.6 IU/ml, 297.3 + 403.5 cells/mm³) and highest in children with moderate persistent (950.24 + 211.65 IU/ml, 337.39 + 321.58 cells/mm³) and later in severe persistent asthma (1000 + 0 IU/ml, 423.87 + 207.86 cells/mm³). And the increasing trend of IgE levels with asthma showed a statistically significant trend ($P =$

0.001 - Kruskal Wallis test – since not following normal distribution), but increasing trend of AEC is not statistically significant, not following normal distribution, values changing widely ($P = 0.151$).

The correlation of total IgE with asthma severity was expressed by use of ROC curve analysis. Tables 2 and Figure 1 explains these relations. Due to small number of study children with severe persistent asthma, ROC curve analysis could not be performed for this patient group. The total IgE cut-off concentration of 168 IU/ml distinguishes children with intermittent asthma from those with mild persistent disease. The IgE concentration of 989 IU/ml distinguishes mild persistent from those with moderate persistent asthma. Both showing adequate or good (Intermittent: mild persistent AUC = 0.952) (mild persistent: moderate persistent AUC = 0.882) diagnostic efficacy. Cut off value of 168 IU/ml may prove useful in practice, indicating that in intermittent group, 75 % of children have serum concentration of total IgE < 168 IU/ml, while 95 % of children in the mild persistent group have total IgE > 168 IU/ml. AEC showed no statistically significant correlation ($P = 0.465$). Similarly, cut-off value of 989 IU/ml, indicated that in the mild persistent group, 87 % of children had serum concentration of total IgE < 989 IU, while 89 % children with moderate persistent asthma had total IgE > 989 IU/ml. AEC showed no statistically significant correlation ($P = 0.206$).

As IgE increases with severity of asthma, absolute eosinophil count also increases. Positive statistically significant correlation is there with changing IgE trend and AEC trend ($P = 0.011$, not following normal distribution).

There is no change in IgE levels or AEC in children with asthma and urticaria ($P = 0.581$, $P = 0.689$ respectively). But there is positive correlation between IgE levels with atopic dermatitis ($P = 0.028$). But not related to AEC ($P = 0.058$). IgE levels and AEC are not showing an increasing trend when the child has both wheeze and allergic rhinitis, ($P = 0.675$, $P = 0.601$ respectively).

CONCLUSIONS

Asthma is highly prevalent in paediatric asthma population. A knowledge about the allergy markers is helpful for the paediatrician to decide for further management of asthma according to standard guidelines.¹ Mean value of IgE and AEC showed increasing trend with severity of asthma in the present study. Associated urticaria, atopic dermatitis and allergic rhinitis with bronchial asthma in children varies the severity of the illness as evident by the increasing trend of IgE levels in these subsets of children. An elevated IgE level and absolute eosinophil count can be considered as a hallmark of atopic sensitization in children and is often associated with symptoms of bronchial asthma.

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