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Role of Three Different Laboratory Tests in Demonstrating Sensitization to Various Allergens in Common Atopic Disorders

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Authors' contributions

This work was carried out in collaboration between all authors. Author SF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript and managed literature searches. Author NMA referred clinically diagnosed cases for laboratory investigation and did literature search. Author FF performed the absolute eosinophil count in pathology department and did literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To study the role of Serum total IgE, serum allergen-specific IgE and Absolute eosinophil count in demonstrating sensitization to various allergens in common atopic disorders.
Materials and Methods: A total of sixty one cases with history of atopy in the form of allergic rhinitis, asthma, and urticaria/ dermatitis were subjected to allergy profile test during a period of one year from January 2013- January 2014. The allergy profile test included serum total IgE, serum allergen specific IgE and absolute eosinophil count.

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Results: In the present study more males (54%) were symptomatic than females (46%). The male to female ratio was 1.17 and overall mean age of atopic patients was 33.73±19.47. The mean age of males in the study was 29.0±20.89 and females 39.32±16.29. Total serum IgE and absolute eosinophil count was raised in 66% and 61% of cases respectively. Serum allergen specific IgE could be performed on 61% of cases and 30% of them were positive. The mean total serum IgE levels correlated well with the clinical symptoms of allergic rhinitis, asthma and dermatitis. Allergen specific IgE results revealed that people were more sensitized to inhalants (54%) than to food items (18%). Around a third of cases (27%) exhibited sensitization to both food and inhalants simultaneously. Moreover, those who were sensitized to inhalants the predominant allergen noted was dust mite (67%) followed by animal and epithelia mix (33%). No such predominance was observed in for food allergens, but subjects showed proportionate amount of sensitization to onion and garlic mix and sea food as 50% each. Further more in Allergodip^{R} test 64% of the subjects showed very high and high grade sensitization of the class 3-4. There was 82% concordance between serum allergen specific IgE results and total serum IgE. Absolute eosinophil count is a more useful predictor of allergic rhinitis than asthma and dermatitis.

Conclusion: The findings suggest that in developing countries with increasing prevalence of atopic disorders, tests like total serum IgE, absolute eosinophil count followed by allergen-specific IgE are helpful in demonstrating sensitization to various allergens if *in vivo* and *ex vivo* tests are not possible for the clinical diagnosis.

Keywords: Atopic diseases; serum total IgE; serum allergen specific IgE; Allergodip ^{R}; inhalants; food allergens; absolute eosinophil count.

ABBREVIATIONS

IgE – Immunoglobulin E
AEC – Absolute Eosinophil Count

1. INTRODUCTION

In the past three decades we have noticed a substantial increase in the allergic disorders [1]. There are several factors associated with this increase but of prime importance are ever increasing levels of environmental pollutants, changing food habits of the people besides, the individuals genetic predisposition to atopy [2]. Nevertheless improved knowledge on immunological aspects of the disease among the scientific fraternity and the availability of novel and highly sophisticated laboratory tests for diagnosis has added to it. In the last but not the least the increased levels of awareness in common man about the allergic diseases.

Earlier we had hardly any test for diagnosis of atopy but now a big panel of tests is available at a hand stretch of allergologist to aid in diagnosis. Especially the Allergodip^{R} from Omega diagnostics UK for detection of allergen specific IgE using dipstick method has simplified the technology to the maximum. Instead of relying on the traditional test requiring special equipment and trained staff, this test can be performed manually without the need of technical expertise and expensive equipment. Moreover, it is not time consuming and painful as ELISA and the skin prick test. The sensitivity and specificity of the test equals to that of the skin prick test and Pharmacia CAP^{R}. Further it has got the added advantage of being performed even when the patient is on antihistamine medication or suffering from atopic dermatitis/ eczema where the skin prick test cannot be done.

1.1 Aim

The present study was done to assess the role of three different laboratory tests, serum total IgE, serum allergen specific IgE [Allergodip^{R}] and absolute eosinophil count in demonstrating sensitization to allergens in common atopic disorders like allergic rhinitis, asthma and atopic dermatitis.

2. MATERIALS AND METHODS

A cross sectional study on sixty one patients with history and clinical sign and symptoms of atopic disease was carried out during the period of one year between January 2013 – January 2014. Individuals were subjected to the allergy profile tests which included

1. Total serum IgE Quantitative assay using Omega diagnostic kit [UK].
2. Serum allergen specific IgE using Allergodip ^{R} test for inhalants and food from Omega diagnostics [UK].

3. Absolute eosinophil count by indirect method.

Blood was collected under aseptic precautions in EDTA and Gel tubes from BD vacutainers US as per the manufacturer instructions.

Patients who have received corticosteroid therapy and antihistamines in the recent past were excluded from the tests.

Total serum IgE was estimated using the quantitative assay from omega diagnostic with the product code GD09 working on the principle of sandwich ELISA. The assay is calibrated against the second international reference serum 75/502 IU/ml. An elevated serum total IgE is strongly suggestive of an atopic predisposition. Hence measurement of total serum IgE is most helpful in diagnosis of atopic patients. Values up to 120/IU/ml are seen in normal individuals and above it are seen in atopic persons. As per the kit insert the normal serum total IgE levels for adults is 188 IU/ml, in children less than ten years it is < 144 IU/ml and in children less than 5 years it is < 135U/ml. This is an age related reference range which varies with geographic locations. There are certain limitations attached with the test, like the test has to be interpreted in conjunction with clinical findings and patient history. The response to allergen is short lived. Patients who did not have a recent atopic challenge may show low IgE levels.

2.1 Allergodip ^{R} EIA

Is a semi quantitative EIA (Enzyme immunoassay) for serum allergen specific IgE. It is relatively simple to use and can be done in absence of any trained technical staff and without the need of sophisticated equipment. Its uses include diagnoses of type I atopic diseases like asthma, hay fever and allergic dermatitis against food allergens. The test is particularly useful for patients in whom skin prick test does not lead to reliable results example during antihistamine therapy, persisting dermatitis or unsuitable skin.

The list of allergens detected in inhalants and food as follows

In inhalation India- sensitization to nine different aeroallergens like corn, dermatophagoides pteronyssinus, Aspergillus fumigatus, eucalyptus globulus, mould mix, grass mix, weed mix, epithelia mix and animal mix is checked.

In food India - sensitization to nine different food materials like cow's milk, wheat four, hen's egg, peanut, chocolate, fish /crustacean, legume mix, cereal/lentil mix and onion/garlic mix is checked.

Principle – Is a solid phase immune assay with allergen covalently bound to carrier molecule on the pad or dipstick. On incubation with serum or plasma the allergen specific IgE binds to the allergen on the pad. After washing to remove the unbound antibody an enzyme labeled anti human IgE is added to link with the human IgE on the pad. Finally a substrate is added and incubated which in case of positive result will give a deep blue color which is evaluated visually. The intensity of the color is directly proportional to the concentration of the allergen specific IgE in the serum /plasma. The results are graded from class 0-4.

0- Not detected
1- Low
2- Medium
3- High
4- Very high

The diagnostic specificity of the test is 100% and sensitivity is 97.5%. Quality control – each dipstick has an inbuilt positive and negative control band which validates the result [The results are semi-quantitative and show a very good - concordance with EAST/CAP (86–97%) and also with skin test (87–98%) [3]. Further the kit performance was validated using external quality assurance samples from UK-NEQAS.

Limitations – Negative results are obtained when the symptoms are not IgE mediated. When samples are taken before individual can produce specific antibody response against the allergen or when IgE levels have reached a minimum level due to long gap after sensitization. Food allergy results are negative in most occasions though the symptoms may be marked. The reason could be due to loss of structural configuration of allergen during industrial processing, cooking or frying. Hence protein structure may not be the same what is in vivo and *in vitro*.

Absolute eosinophil count was performed using the indirect method. In indirect method both the total leukocyte count and differential leukocyte count are first estimated. Then absolute eosinophil count is calculated by finding the product of the two i.e. the eosinophil percentage and the total leukocyte count [4]. Levels above 400 cells /cumm indicate eosinophilia.

2.2 Statistical Analysis

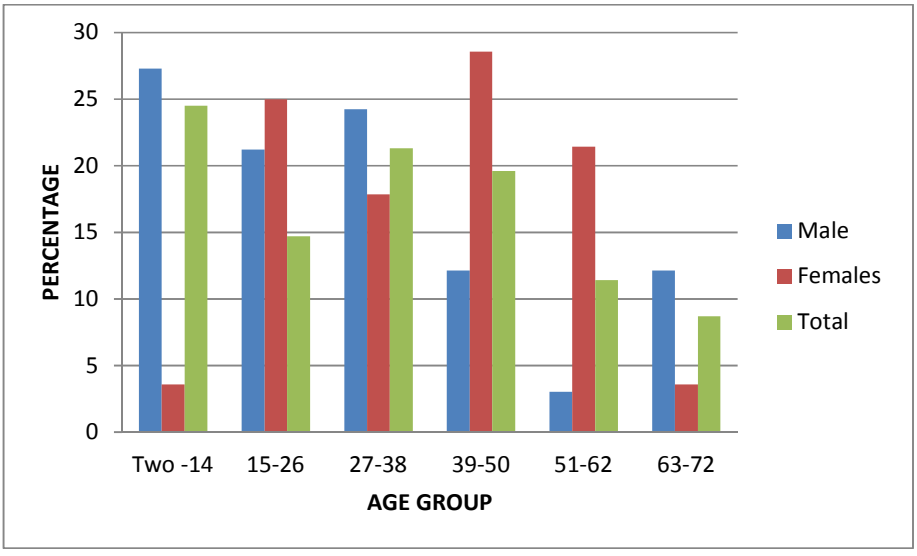
Data was analysed using the soft ware EPI INFO 7.0 from CDC [Centers for disease control and prevention].

3. RESULTS

The present study included 61 subjects with history of various atopic disorders like allergic asthma as 14, allergic rhinitis as 39 and atopic dermatitis as 8 cases. Majority of the subjects were males 54% followed by 46% females. The male to female ratios was 1.17 and overall mean age for the test subjects noted was 33.73±19.4. Males were symptomatic at an earlier age of 29.00±20.89 than females 39.32±16.29 with a significant *P* value of 0.035. Total serum IgE and absolute eosinophil count were raised in 66% and 61% of the cases respectively. In the present study the mean serum total IgE level noticed was 239.414±221.458 which is above the expected normal value of 120 IU/l. In males it was noted as 239.867±262.726 and in females it was seen as 233.3540±150.94. Moreover, the mean total serum IgE in individuals with sensitization to inhalants was higher as 345.7±270.81 than in subjects with sensitization to food items as 260.37±30.93 and in individuals with simultaneous sensitization to both inhalants and food the value seen was 158.70 ± 142.2. Hence it is evident that total serum IgE response is higher to aeroallergens than to food allergens. Overall the serum total IgE levels had a good correlation with the results of serum allergen specific Ig E corresponding to 82% of the total 11 patients in whom serum allergen specific IgE was detected; 82% of them had raised total serum IgE levels in the range of 200 IU/ml- 913.487 IU/ml respectively.

Serum allergen specific IgE test using Allergodip[R] for food and inhalants [India] revealed that 30% of the tested individuals showed sensitization and presence of allergen specific IgE in their serum either to food items, inhalants or to both. More number of subjects were sensitized to inhalants (54%) than to food items (18%). Some individuals showed simultaneous sensitization to both i.e. food items and inhalants as 27%. In individuals with sensitization to inhalants the predominant type of allergen noted was dust mite in 67% followed by sensitization to allergens like animal and epithelia mix 33%. In food allergens we have noticed an equal proportion of subjects sensitized to onion and garlic mix and sea food as 50% each. Twenty seven percent of the subjects exhibited simultaneous sensitization to both dust mite and sea food together. Furthermore, around 64% of the subjects showed very high and high grade of sensitization to the allergens tested rest 36% of the subjects exhibited medium and low level of sensitization.

The studied subjects were in the age group 2-72 years. Majority of the symptomatic subjects were in the age group 15-62 years as 67%; rest 33% were in the age group of two extreme ends of the graph, as seen in Graph 1.

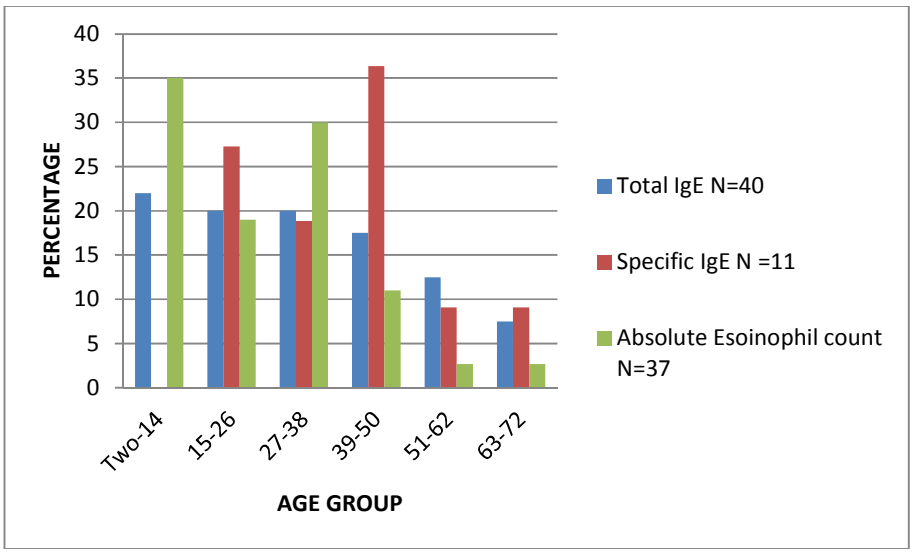


Graph 1. Age and gender wise distribution of test subjects

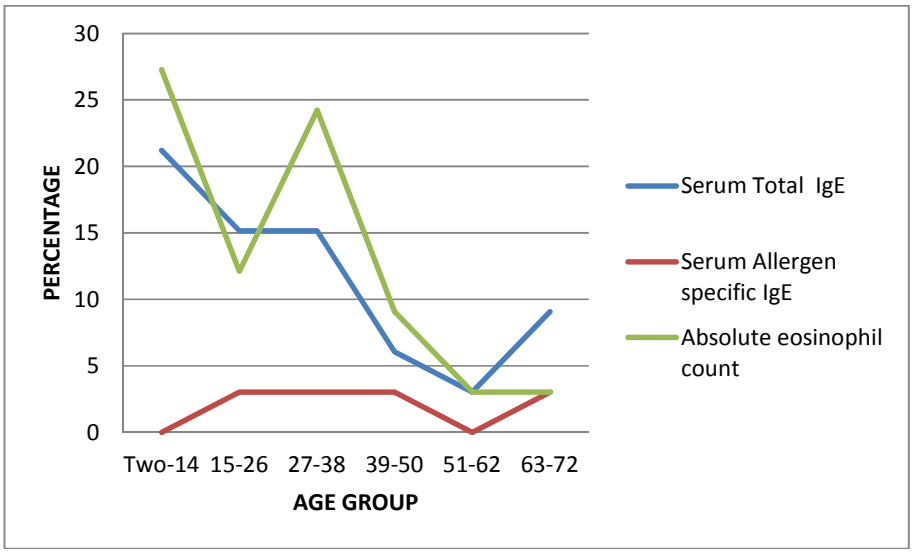
Serum total IgE levels and absolute eosinophil count were noted to be raised in maximum number of individuals in the age group 2-14 years and declined with the age. Serum allergen specific IgE had a characteristic pattern of distribution between the age group 15- 72 years with maximum number of subjects in the age group 39-50 years as seen in Graph 2.

With respect to gender more number of males were clinically symptomatic (54%) than females (46%). These findings reflected even in the results for total serum IgE and AEC as 23/40 (58%) and as 26/37 (70%) were men. On the other side the serum allergen specific IgE test was detected in more number of females as 7/11 (64%) than in males.

Males were symptomatic at an earlier age to atopic diseases as seen in Graph 1. Serum total IgE and AEC tests showed a characteristic distribution in males with maximum number of subjects in the age group of 2-14 years which gradually declined as the age increased as seen in Graph 3. Further all the three tests were seen to be positive in majority of the males in the age group 27-38 years as seen in Graph 3.



Graph 2. Age wise response to three different tests in study



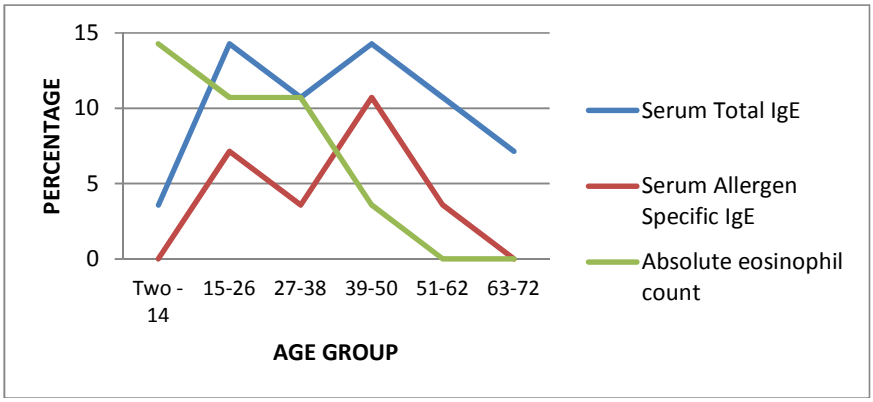
Graph 3. Age wise distribution of three tests results in males

Majority of the females were symptomatic and gave a positive response to all the three tests in a wider age group range of 15-62 years as seen in Graphs 1 and 4. Hypereosinophilia was detected in more females in the age group 2-14 years. But serum total IgE was raised and serum allergen specific IgE detected in majority of females in the age group 15-62 years. Furthermore, we can say a biphasic response to these two tests was observed within this wider age group of 15-62 years with maximum numbers detected in 15-26 years of age followed by 39-50 years with a slight dip in the curve in the age group 27-38 years. The numbers then declined at the other two extremes of age that is 2-14 years and 63-72 year. Serum allergen specific IgE was not at all detected at the two extremes of age in females as seen in Graph 4.

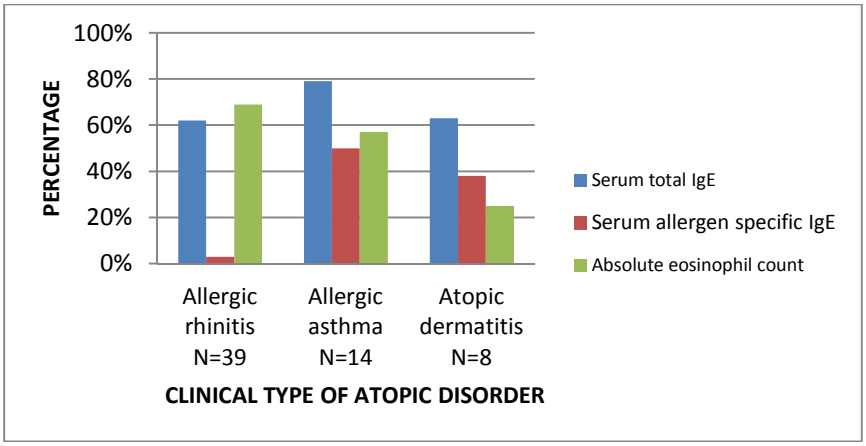
Therefore from the results it is clear that hyper eosinophilia as a marker of sensitization to various allergens occurred in both the sexes at an earlier age. Serum total IgE levels correlated with symptoms in males at a younger age group but not so in females. Serum allergen specific IgE was detected in both the sexes in higher age group as seen in Graphs 2, 3 and 4.

When the three different tests were compared against the clinical presentation of the subjects; the total serum IgE levels and serum allergen specific IgE were significantly elevated more so in asthma as 79% and 50% cases followed by dermatitis as 63% and 38% and least in allergic rhinitis as 62% and 3% . On the other hand AEC was raised in more number of allergic rhinitis (69%) cases, followed by asthma (57%) cases and dermatitis (25%) cases as seen in the Graph 5.

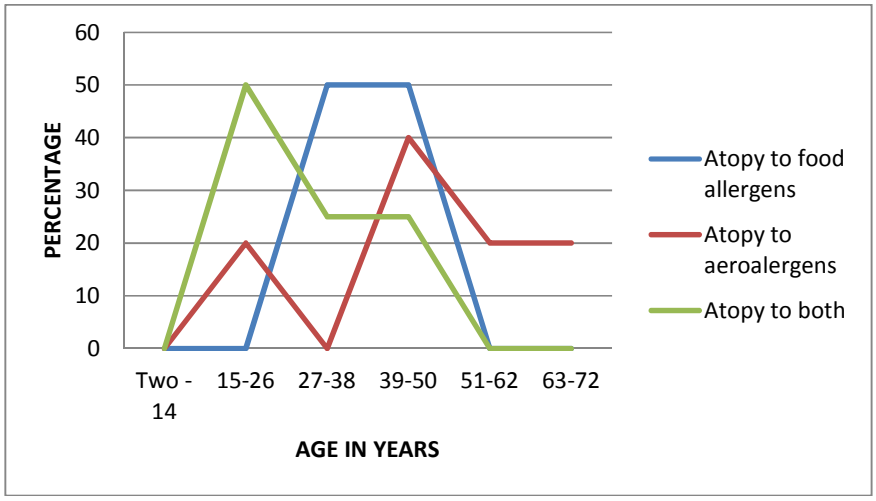
In the present study maximum sensitization to food allergens was noticed in the age group 27 to 50 years; whereas for inhalants it occurred in the age group between 39-50 years and sensitization to both the types of common allergens was seen in the age of 15-26 years which is evident from the Graph 6.



Graph 4. Age wise distribution of three tests results in females



Graph 5. Utility of three different tests in detecting sensitization



Graph 6. Age wise distribution of atopy to different allergens studied

4. DISCUSSION

The main stay for diagnosis of atopic disorders is a good clinical history of exposure to the allergen, skin prick test and in vivo or ex vivo provocation tests. Blood tests which includes total serum IgE and peripheral eosinophil count aids in diagnosis [5,6]. Several authors have opined that serum total IgE levels are of low predictive value in diagnosis of atopy than the specific IgE [5]. But at the same time many have demonstrated its utility as a good predictor of atopy [7]. Independently also the serum total IgE levels have a good predictive value in demonstrating sensitization [8,9].

In the present study total serum IgE was elevated in 66% of the subjects which is equal to one reported by Satwani H et al. as 65%, and lower when compared to the results reported by Ahmed I et al as 70%, Al Shami et al as 82.9% and Somani VK as 88% [10-12]. Nevertheless, irrespective of the clinical type of atopy, serum total IgE was significantly elevated in substantial number of atopic patients justifying the statement that total serum IgE is the hall mark of atopy or a significant marker of atopic state as mentioned by Ebrahim razi et al and Sudha deo et al. [13-14]. Furthermore as stated by Jagadeeshwar et al. and others that at least 50% of the individuals with atopy have serum total IgE concentrations raised two standard deviations above the mean normal control group holds true [15-18]. Therefore we conclude saying that it is a reliable and cost effective indicator of atopic sensitization. In the present study it is observed that serum total IgE levels correlated well with allergen specific IgE findings in 82% of the subjects which is consistent with the reports by [7,11].

Hypereosinophilia indicates an inflammatory process and is seen in parasitic infections and various allergic states. Reports on association of eosinophil counts or its percentage in various atopic states is debated. Some have found it to be very informative along with serum total IgE in subjects with allergic rhinitis than in other allergic states [11,15]. Others have reported it to be of no relevance even in allergic rhinitis as well [11,19]. Moreover, some author has reported it to be of significance not only in diagnosis of allergic rhinitis but also in monitoring the course of the disease and its management [20-24]. Our results for absolute eosinophil count showed it as the primary indicator of sensitization in children with maximum results in the younger age group of 2-14 years in both the sexes. Furthermore the eosinophil counts correlated well with symptoms of allergic rhinitis as 69% were having high counts than with asthma 57% and dermatitis 25%. This is in agreement with the findings of [7,15].

Detection of serum allergen specific IgE is not possible in all cases because of cost constraint as is the case in our study though it is a useful marker of sensitization or atopic response [11,15]. Here the allergen Specific IgE or Allergodip ® test was detected in 30% of the subjects whereas others have found it in 65% of the patients [12]. The results of serum allergen specific IgE were more consistent with asthma as 50% of them showed sensitization, than with

dermatitis 38% and allergic rhinitis 3% cases. [15]. Majority of the subjects showed sensitization to inhalants than to food items which is similar to the reports by [12,14]. The most common aeroallergens noted in the present study are dust mite as 54% followed by epithelia mix and animal mix 46%, which is similar to the reports by Darsow U et al. as 56% for dust mite and 49% for animal and epithelia mix [12,14,25]. Sensitization to food items was seen in only 18% of the individuals, which is less when compared to the one reported by Burkes et al as 33%, 37% by Eigenmann PA et al. and 51% by Niggemann et al. [26-28]. The reason for low detection rates for food allergens in the present study could be same as mentioned by others; as loss of allergen specificity during processing, highly unstable nature of food allergen when compared to aeroallergens and the methods used in extraction which influences its structural integrity. Therefore there are more false negatives results despite strong symptoms as mentioned by Straumann F et al. [29]. The most common food allergens detected were sea food and onion and garlic mix, which is similar to the one reported by [30,31]. The total IgE response was also significantly low in these subjects when compared to subjects with sensitization to inhalants. Serum allergen specific IgE results were more consistent with asthma than with dermatitis and allergic rhinitis. Furthermore, of the 30% subject's with sensitization to specific IgE, 64% had very high and high grade sensitization of class 3-4 level.

With regards to variables like age and sex studied more number of males were symptomatic for atopy than females which correlates with the reports of [10,11,13-15].The mean age of the patients suffering with atopy in our study was 33.73±19.47 which is in consistent with the study done by [15]. Males were symptomatic at an earlier age than females indicating a most probable role of genetic predisposition as reported by others [14,15,18,23]. Males gave a maximum response to the all the three tests in the age group of 27- 38 years where as females showed a similar response in a wider age group range of 15-62 years [32].

Total serum IgE levels and absolute eosinophil count showed a characteristic age specific decline in numbers of positive subjects from 2-72 years of age with a maximum percentage in the age group 2- 14 years which gradually decreased as the age progressed.

Inspite of the attached limitations total serum IgE can be used as a good predictor of sensitization as reported by many authors they have recommended its use in cases with predominant symptoms of atopy [12,18]. Furthermore some authors have even correlated high total IgE levels with the severity of the disease too .They were of the opinion that in patients with various clinical types of asthma and allergic rhinitis the serum total IgE levels not only correlated with the degree of severity of the disease too but even response to treatment as well [20-24,33-34] but our study was unable to demonstrate this response.

On the contrary to serum total IgE and AEC, serum allergen specific IgE was detected in more number of females as seen in study by Halonen et al 1982 [32]. Sensitization to aeroallergens as reported by other authors was common in the older age group 39-50 years when compared to food allergens which is seen in the age group of 27-38 years and response to both the allergens at a still younger age group of 15-26 years [13].

5. CONCLUSION

Diagnosis of allergic diseases is performed upon a good clinical history ideally based on an appropriate selection of diagnostic tests that do not overburden the patients with financial issues. The skin allergic tests and the *in vivo* and *ex vivo* provocation tests play ideal role, but when there are inexpensive and painless techniques of equal sensitivity and specificity available to demonstrate specific hypersensitivity to airborne and/or food allergens [35,36]. Our findings suggest that total serum IgE and AEC are useful for screening sensitization and allergen-specific IgE a reliable tool when skin tests are not possible.

6. LIMITATIONS

The present study has got limitations like the study population was small. Secondly the serum allergen specific IgE could not be performed on all subjects due to cost constraints. Finally we could not group the study subjects based on severity of the atopic symptoms and compare the results of test response due to lack of clinical data.

CONSENT

An informed oral consent was collected from all the subjects in the study prior to testing.

ETHICAL APPROVAL

Prior permission was obtained from institutional ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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