Role of Ige Elisa Testing in Diagnosis of Allergic Reactions: Study in Tertiary Care Hospital of Western Uttar Pradesh

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ABSTRACT

Introduction: Immunoglobulin E (IgE) occurs in extremely low amounts in blood and tissues but is the key immunoglobulin in allergy. The quantification of allergen-specific IgE levels (sIgE) in serum and body fluids is important because sIgE concentrations are useful to define clinical phenotypes of allergy and to predict the intensity of allergic reactions.

Aim & objectives: The purpose of this study was to analyse the role of IgE ELISA for diagnosis of allergy in patients with respiratory and skin allergies.

Material and Methods: Study comprises of 147 patients, with clinically suspected allergic reactions. The serum samples was taken and subjected to ELISA.

Results: Among the 147 patients, who fulfilled the inclusion criteria were screened for IgE ELISA testing, 114 (77.5%) were found to have raise in IgE levels. The raise in IgE levels was predominantly seen in females (94) as compared to male cases (53).

Conclusion: The presence of specific IgE is an indicator of sensitization to allergens. Identification of specific allergens in allergic patients enhances management through education, allergen avoidance, and immunotherapy, each of which can improve clinical outcomes.

Keywords: Type 1 Hypersensitivity, Immunoglobulin E(IgE), IgE ELISA

Introduction

Type I hypersensitivities include atopic diseases, which are an exaggerated IgE mediated immune responses (i.e., allergic asthma, rhinitis, conjunctivitis, and dermatitis), and allergic diseases, which are immune responses to foreign allergens (i.e. anaphylaxis, urticaria,

angioedema, food, and drug allergies). The allergens that result in a type I hypersensitivity may be harmless (i.e. pollen, mites, or foods, drugs, etc.) or more hazardous such as insect venoms.[1] The reaction may be manifested in different areas of the body and may result in instances such as: Nasal allergic rhinitis or hay fever, ocular allergic conjunctivitis, potentially due to seasonal allergens such as pollen or mold spores, dermatological hives, Atopic Eczema, or Erythema, soft tissue Angioedema, Pulmonary reactions, such as allergic asthma or hypoxia, systemic reaction, which is a life-threatening medical emergency, and also known as Anaphylaxis.

There are certain risk factors that increase the risk of allergic diseases. These factors include geographical distribution, environmental risks such as pollution or socioeconomic status, genetic predisposition, or the "hygiene hypothesis". The "hygiene hypothesis" suggests that our modern society practices of good hygiene and the lack of early exposure to many microbes or antigens may result in failures of the immune system functionality. As such, the hypothesis suggests that early exposure to a diverse range of microorganisms and antigens may actually lead to overall decreased rates of allergies, asthma, and other immune disorders.[2][3][4]Allergy is the most common immunologically mediated hypersensitivity disease worldwide affecting upper and lower respiratory tract, the skin as well as the gastrointestinal tract and may cause life-threatening anaphylaxis.[5]

Immunoglobulin E (IgE) occurs in extremely low amounts in blood and tissues but is the key immunoglobulin in allergy. The quantification of allergen-specific IgE levels (sIgE) in serum and body fluids is important because sIgE concentrations are useful to define clinical phenotypes of allergy and to predict the intensity of allergic reactions.[6,7,8,9]Understanding the role of IgE in allergic reaction has been a major breakthrough in the field of allergy. It has paved the way for the discovery of effective drugs for these diseases.

The sequence of events in the allergic reaction consists of the production of IgE antibodies in response to an allergen, binding of IgE to Fc receptors of mast cells, cross-linking of the bound IgE by the allergen upon re-exposure, and release of mast cell mediators such as histamine, lipid mediators and cytokines. Some mast cell mediators cause rapid increase in vascular permeability and smooth muscle contraction, resulting in many of the symptoms.[10, 11]

The purpose of this study was to analyse the role of IgE ELISA for diagnosis of allergy in patients with respiratory and skin allergies.Lack of data in this context, particularly from this geographical area prompted us to carry out this study

METHODOLOGY

This study was conducted in department of Microbiology, LLRM Medical College in collaboration with Department of Dermatology and Department of Respiratory Medicine for the period of one year from January 2022 to December 2022.All patients of either age, both male and female coming to Dermatology OPD with skin manifestations and Respiratory OPD with suspected allergic reactions were included in the study. A total of 147 patients with allergic reactions and suspected raise in IgE levels, aged from 5 to 95 years, were included in the study and tested by IgE conventional ELISA using Calbiotech IgE Elisa Kit and Robonik Elisa Reader.

Blood specimens were collected and serum was separated.

REAGENTS PREPARATION: Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature i.e. 20-25^{0}C.

ASSAY PROCEDURE

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder

2. Pipette 25 µl of IgE standards, controls, and samples into appropriate wells.

3. Add 100 µl of Biotin Reagent into each well. Shake the plate for 10-30 sec.

4. Cover the plate and incubate for 30 minutes at room temperature (20-25^{0}C).

5. Remove liquid from all wells. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.

6. Add 100 µl of Enzyme Reagent into each well.

7. Cover the plate and incubate for 30 minutes at room temperature (20-25^{0}C).

8. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.

9. Add 100 µl of TMB substrate to all wells.

10.Incubate for 15 minutes at room temperature.

11. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.

12.Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. Check IgE standard value on each standard vial. This value might vary from lot to lot.
2. To construct the standard curve, plot the absorbance for the IgE standards (vertical axis) against its concentration in IU/ml (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Use the absorbance for controls and each unknown sample to determine the corresponding concentration of IgE from the standard curve.

RESULT

Among the 147 patients who fulfilled the inclusion criteria were screened for IgE ELISA testing, 114 (77.5%) were found to have raise in IgE levels as shown in Figure 1.

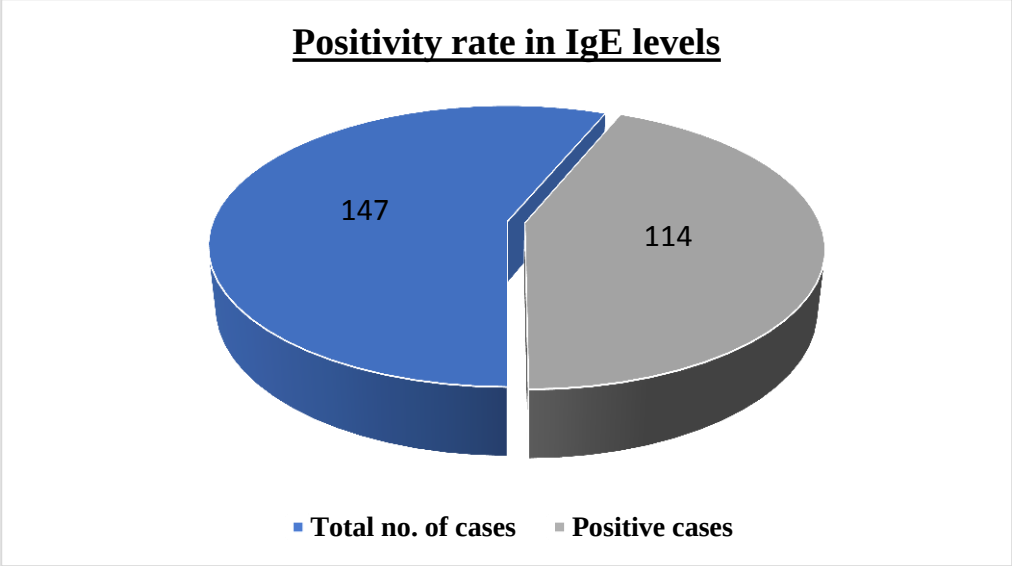

Figure 1: Shows the Positivity rate of IgE levels

The raise in IgE levels was predominantly seen in females (94) as compared to male cases (53) as depicted in Figure 2.

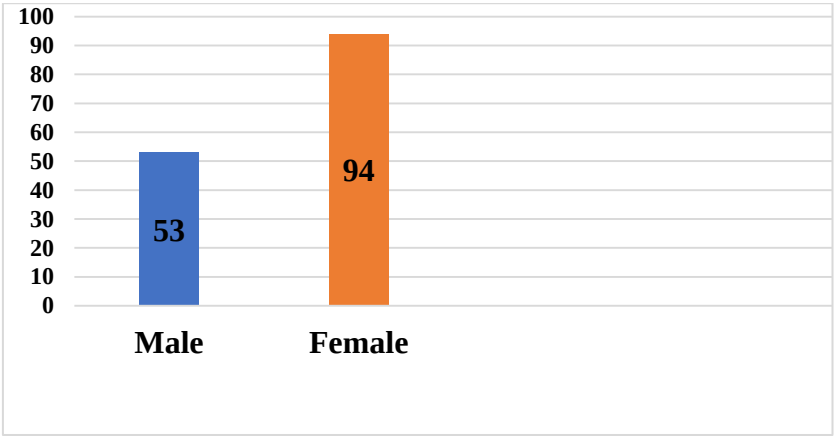

Figure2: Gender wise distribution of raise in IgE levels

The raise in IgE levels were seen in the month of April, September and October as depicted in Figure 3.

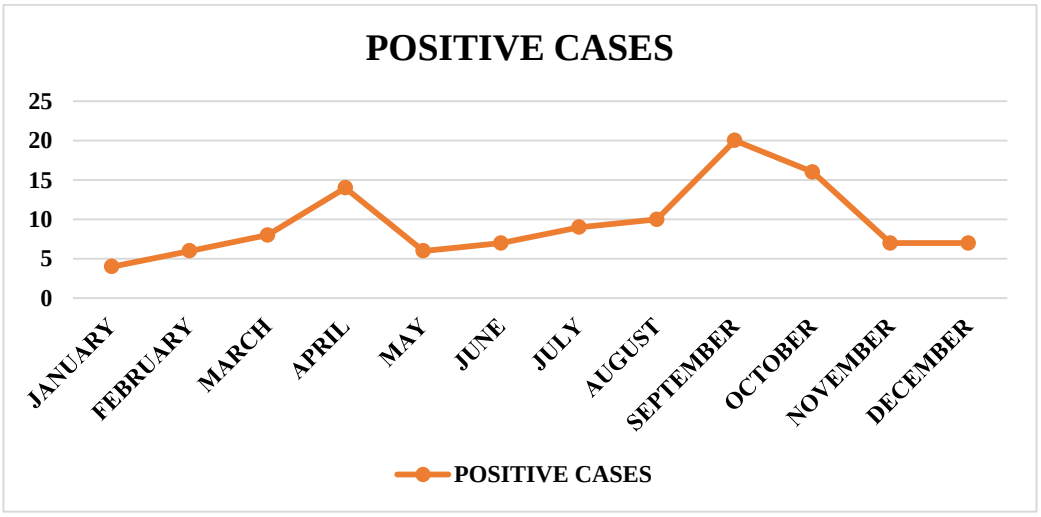

Figure 3: Month-Wise Distribution Of Raise In Ige Levels

The maximum number of cases was seen from the patients with skin allergies as compared to respiratory cases as shown in figure 4.

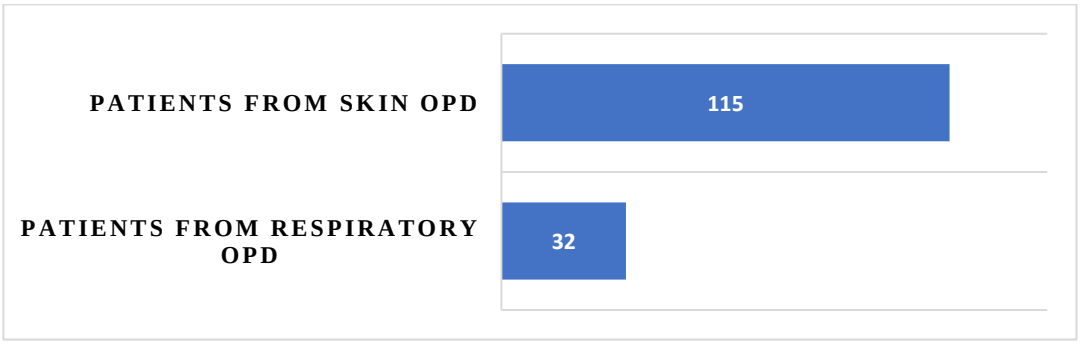

Figure 4:Comparison between respiratory and skin manifestations

DISCUSSION&CONCLUSION

Allergic diseases are complex diseases arising from interactions between genetic and environmental factors. The detection of allergen-specific IgE is an important method for the diagnosis of IgE-mediated allergic diseases.[16]In this study, the positivity rate for cases screened by IgE ELISA was 77.5%. Similarly, Ritter et al.,[13] and Atta et al.,[12] has reported a positivity rate of 43.6% and 75%in an allergic infection respectively.

In our study, females (82.4%) outnumbered males (46.4%) in allergic reaction cases. Johnson et al.,[15]also reported a similar female predominance in 79.4% of the cases.In our study, there was low case load in month of December and January with rise in May to October. Concordant results were obtained in a study done by Barbee et al.,[14]. Thus, it can be concluded that the presence of specific IgE is an indicator of sensitization to allergens. Identification of specific allergens in allergic patients enhances management through education, allergen avoidance, and immunotherapy, each of which can improve clinical outcomes.

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