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Concordance and relevance of total and specific IgE allergy laboratory assays at a major Jordanian hospital

Ababneh Hani MD

Immunology Department at King Hussein Medical center (K.H.M.C) Amman Jordan

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ABSTRACT

Objective: In vitro allergy diagnostic testing is becoming increasingly common in our area. This study was conducted to correlate and evaluate the concordance between total IgE measurement and specific IgE radioallergosorbent test (RAST) allergy assays. **Methods:** A total number of 2323 sera samples were received over a five years period (1-1-2007 till 1-1-2012) to perform allergy screen, of which 1627 were analyzed for total IgE only, 969 for RAST only, and 129 for both assays as requested. Total IgE test was performed by enzyme-linked immunosorbent assay (ELISA) and RAST by immunoplot method. **Results:** A total number of 129 sera samples received for RAST and total IgE testing, were analyzed for both; the results were categorized into 3 groups according to the total IgE level. <20 iu/ml, 20-100 iu/ml, and >100 iu/ml. Of the 47 (36.4%) samples which composed the first group with IgE <20 iu/ml, 2 (1.6%) were positive only for RAST showing the lowest concordance. With no need for performing the RAST test unless the clinical picture was suggestive of allergy and the skin prick test was positive. Of the 30 (23.3%) samples which composed the second group with IgE 20-100 iu/ml, 6 (4.7%) were positive for RAST (Chi-square = 4.8757 and P-value = 0.0272). Of the 52 (40.3%) samples which composed the third group with IgE >100 iu/ml, 19 (14.7%) were significantly positive for RAST compared to the second group (Chi-square 15.3948 and P-value = 0.0001). By the use of regression, RAST positive increased with increasing IgE level (direct proportion) (Pearson Correlation (R) = 0.347, P-value < 0.0001). **Conclusion:** Total IgE appears to be useful in predicting positive results in allergen specific IgE tests to common allergens. The specific IgEs to certain aero and food allergens showed good correlation with total IgE. Few articles in our area have been published about laboratory testing for allergy, so our findings will enrich the literature database of the area with updated information of the large number of samples being delivered to KHMC, one of the major hospitals in the region. We will not recommend performing the RAST test unless the clinical picture is suggestive of allergy and with a positive skin prick test, for better cost effectiveness, since Jordan is developing country with limited resources.

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1.Introduction:

Allergic diseases are among the most common chronic conditions in the adult and pediatric population. Approximately 40% of the world's population and 30% of the Jordanian population suffer from allergic diseases, with escalating prevalence to epidemic proportions. IgE is an immunoglobulin

that's made of two identical light chains and two identical ϵ chains that are linked by a disulfide bond, the ϵ chain has one variable region and four constant regions, and the light chain has one variable region and one constant region

Allergic rhinitis, which is a risk factor for asthma, affects 400 million people, and food allergies affect about 200 to 250 million people annually. An estimated 250,000 avoidable deaths from asthma occur each year. Allergy diagnostic testing is an important part of the evaluation/management of allergic patients because the history may not be precise enough to identify the specific

* Corresponding Author : Dr. R. Khasawneh (KHMC),
E-mail: dr_r_khasawneh@yahoo.com

allergen, Allergen-specific IgE antibody assays are designed to detect and measure circulating IgE antibodies that can bind to specific allergens. The ability of the assay to detect allergen-specific IgE is dependent on several factors, including the quality of the allergen component,

All allergic Type I-reactions are preceded by an initial contact phase without symptoms during which Class E specific antibodies (IgE antibodies) are formed. On repeated contact with the allergens which trigger the reaction, these IgE antibodies react with the allergens and lead to the release of mediators (usually from mast cells or mastocytes) such as histamine, leucotrienes and prostaglandins, which lead to the symptoms of allergy.

Three diagnostic panels were used including three allergen Panels

Inhalant panel is especially useful in patients with allergies to inhaled allergens. They are comprised of pollen allergens found in different geographic regions of Jordan. Pollen allergens are grouped by class: grasses, trees, and weeds.

Food allergen panel is grouped by food type, including dairy, fish, grains, meats, nuts, and seafood. Pediatric allergen panel including cow milk

When carrying out allergy diagnostics, the determination of increased total IgE indicates the presence of sensitization or allergy. The highest IgE values are found with atopic dermatitis and may reach in excess of 20,000 IU/ml. Although IgE is present in trace amounts in serum it can be greatly increased in allergic people and parasitic infections such as *Plasmodium falciparum* strongyloids, *Schistosoma mansoni* and infectious diseases such as mycoplasma infections, whooping cough, HIV and malignant myelomas or even immune deficiency. Although very low total IgE values indicate that there is no allergy, it cannot be ruled out.

IgE is medically important for two reasons, it plays an important role in the allergy disorder, and is especially associated with type 1 hypersensitivity and it may also signify infection by helminthes and certain other types of parasites as mentioned previously.

RAST is radio allergo absorbent test that is used to measure the amount of the specific IgE in the serum that reacts with a known antigen (allergen)

RAST measurement has approximately similar diagnostic value of that of the skin tests but not affected by symptoms or treatment like antihistamines or anti-inflammatory drugs, it uses blood samples so it's a safe procedure and avoids the risk of anaphylaxis reaction that maybe produced by skin prick testing, it's also the test of choice for patients who have widespread eczema and dermatographism

At our laboratory we have three diagnostic panels respiratory food and pediatrics, which have different allergens including indoor, outdoor, domestic animals, pollens, food, insects, and fungi.

Specific IgE (sIgE) the test is excellent for identification of a sensitized state where specific IgE exists; the result of this test does not correlate with the severity of the clinical reaction. However, the higher the sIgE, the greater the likelihood of clinical allergy Tests for sIgE may be influenced by cross-reactive proteins that may or may not have clinical relevance to disease. Interpretation of RAST results should be correlated with clinical history

2. Materials and Methods

In this retrospective study a total number of 2323 sera samples were received over a five years period (1-1-2007 till 1-1-2012) to perform allergy screen, of which 1627 were analyzed for total IgE only, sent all through the year with the following percentages according to the months in which they were sent : 318(19.5%) in January and February. 541(33.25%) in March, April, May, and June. 321(19.75%) in July, August, and September. 445(27.5%) in October, November, and December. Total IgE was performed by enzyme-linked immunosorbent assay (ELISA) Biopharm.Germany.

IgE was categorized into 3 groups according to the total IgE level <20 IU/ml 524(32.2%), 20-100 IU/ml 513(31.53%), and >100 IU/ml 590(36.27%). The test corresponds to an enzyme immunoassay (EIA) for the determination of antibodies. Anti-human IgE antibodies of non-human origin are bonded to the inner surfaces of the wells of a microwell plate. Human IgE antibodies present in the standards, controls and patient samples are bound by these antibodies. In the second step, an anti-human IgE antibody conjugated with alkaline phosphatase is added which attaches itself to the bound human IgE antibody. In the third step, a substrate is added which is dephosphorylated by the alkaline phosphatase. The resulting color change from colorless to yellow is measured using a photometer at 405 nm and a reference wavelength of 620 nm. The intensity of the yellow color is proportional to the quantity of IgE-antibodies in the serum. The results are expressed in IU/ml

RAST was requested for 969 only, of which 129 (18.53) were positive for specific allergen whether it was in the food, respiratory or pediatrics panel. RAST was performed by Immunoplot Biopharm, Germany, RIDA® X-Screen this is an instrument for measurement and evaluation of allergy Screen immunoblot test membranes including software for documentation and archiving.

The quantitative evaluation of this allergy Screen panels is a high sophisticated tool for the semi-quantitative analysis of specific IgE carried out using an instrument including CCD camera. The reaction trough with the panel membranes will be

inserted into retainer of the screen, following by the measurement using the appertaining PC software. The IU/ml will automatically be calculated from the measured values and assigned to the EAST classes 0-6. The evaluation is based on a standard curve which is saved to each pre-defined test.

Serum dilution: undiluted (or 1: 10); conjugate class anti-IgE (monoclonal), AP-labeled antibodies against up to 36 allergens per strip can be simultaneously nonspecifically detected. The programme Scan from Euroimmun has been developed to enable semi-quantitative evaluation of Euroline analyses, facilitate management of data, and provide detailed documentation of results. The test strips are scanned using a flatbed scanner. Euroline scan recognizes the position of the strips, identifies the bands, and measures their intensity.

Euroline profiles with different allergen compositions are available for various test requirements.

Specific IgE profiles with different allergen compositions are available for various test requirements: atopy, inhalation, food, insect venoms and cross reactions.

Frequency of Allergens Sensitization

Inhalants Allergens	%
HDM	30
Pollen (olive and grass)	28
Cockroach	14
Food	12 (17 3,5)
Cat	9

Food Allergens	%
Milk	29
Nuts , egg	25
Wheat	8
Others	13

Figure 1

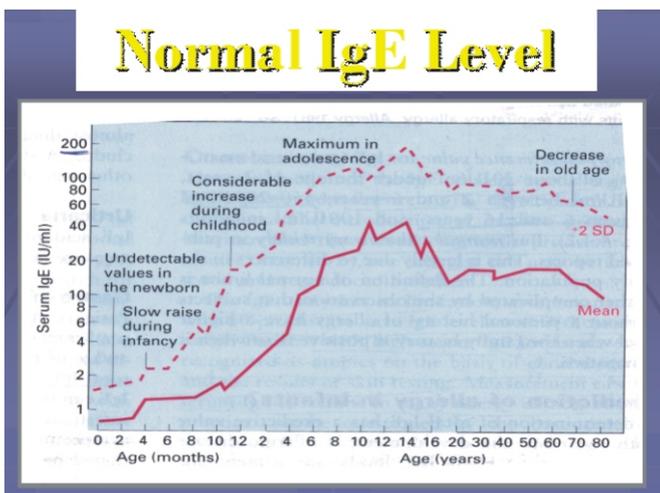
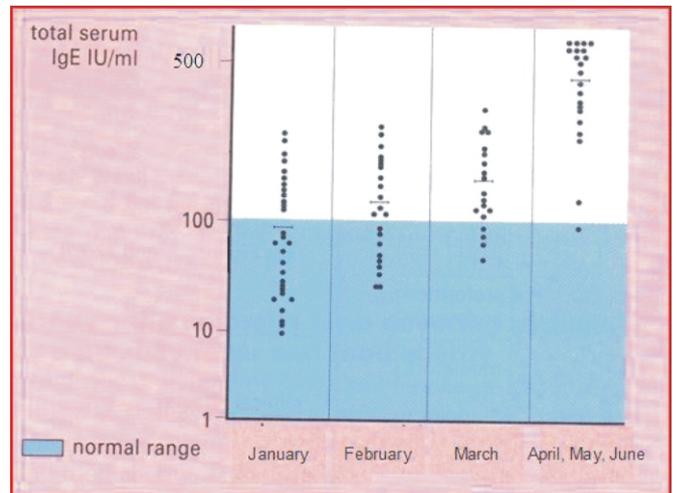


Figure 2: Variation of IgE concentration during the year



3. Results

Of the 1627 sera samples 129 were tested for both assays as requested.

There were no duplicates, and most samples were sent for diagnostic purposes.

Of the total number of 129 sera samples which had RAST and total IgE requested were analyzed, and the results were categorized into 3 groups according to the total IgE level <20 Iu/ml ,20-100 Iu/ml, and >100 Iu/ml.

IgE level <20 Iu/ml were 47 samples of which 45 were negative for RAST and only 2 were positive.

IgE level 20-100 Iu/ml were 30 samples of which 24 were negative for RAST and 6 were positive.

IgE level >100 Iu/ml were 52 samples of which 33 were negative for RAST and 19 positive.

The following data shows the percentage, p-value and chi square of the results:

IgE 20-100 Iu/ml group showed significantly more RAST + than IgE group (Chi-square = 4.8757 and P-value = 0.0272)

IgE >100 Iu/ml group showed significantly more RAST + than IgE 20-100 Iu/ml group (Chi-square 15.3948 and P-value = 0.0001)

Other method

By the use of regression RAST + increased with increasing IgE level (direct proportion) (Pearson Correlation (R) = 0.347, P-value <0.0001). Listing of Available Allergens Of the three groups,

The group with IgE >100 Iu/ml had the most positive RAST results, and the most concordance with p-value of 0.0001 and chi square of 15.3948.

inserted into retainer of the screen, following by the measurement using the appertaining PC software. The IU/ml will automatically be calculated from the measured values and assigned to the EAST classes 0-6. The evaluation is based on a standard curve which is saved to each pre-defined test.

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The group with IgE >100 IU/ml had the most positive RAST results, and the most concordance with p-value of 0.0001 and chi square of 15.3948.

The group of IgE 20-100 IU/ml showed lower concordance with RAST but it was still significant with p-value of 0.0272 and chi square of 4.8757.

The group of IgE <20 IU/ml showed the lowest concordance, and we will not recommend performing RAST testing unless the clinical picture was suggestive of allergy and skin prick testing was positive, for better cost effectiveness, and evidence based investigation to save time and labor. Figure 1 shows the results

4. Discussion

1,200,000 (20 %) of the Jordanians (out of 6,000,000) have allergy, 33% of allergic patients developed asthma Although the total IgE level increase is not specific for a certain allergen or even allergy in itself because it is increased in other settings such as parasites infection, it indicates allergy because IgE plays an important role in the allergy disorder, and is especially associated with type 1 hypersensitivity IgE increases with age reaching high normal concentration in adults Figure 2

Although there are patients with allergies that fall into the normal level, higher levels points to allergy hence knowledge of the total IgE level gives a rough estimate to guide further investigations like RAST testing. Total IgE increased mostly on April, May and June due to flowering and pollen spreading of outdoor grasses. Figure 3

The clinical picture should always be correlated with the results, and if it is suggestive of allergy a normal IgE level doesn't rule out allergy and further testing is required, at the same time in a patient where the clinical picture doesn't correlate with a high IgE level the increase could be attributed to other non allergy causes such as parasitic infection.

RAST is specific IgE and our results of the test are in direct proportion to the total IgE, samples with high total IgE tend to have more positive RAST results, knowledge of the specific allergen helps with management of the disease by avoidance of the allergen to lower frequency, and severity of symptoms.

Less than 10% of the requested tests have a specific allergen requested so we run the whole panel for the sample, which costs more than the specific allergen. Testing for IgE antibodies is not useful in patients previously treated with immunotherapy to determine if residual clinical sensitivity exists, or in patients in whom the medical management does not depend upon identification of allergen specificity.

Some individuals with clinically insignificant sensitivity to allergens may have measurable levels of IgE antibodies in serum, and results must be interpreted in the clinical context.

5. Conclusion

The multiallergen IgE antibody test, combined with measurement of IgE in serum, is a very Sensitive first-order test for allergic disease. Positive results indicate a high probability of allergic disease induced by 1 or more allergens present in the

multiallergen panel. Negative results effectively rule-out allergy, except in rare cases of allergic disease induced by exposure to a single allergen. Panels may be ordered with or without concurrent measurement of IgE. Measurement of the concentration of IgE in serum is useful in the initial evaluation of patients Suspected of having an allergic disease.

Concentrations measurement of serum IgE along with the multiallergen IgE antibody test offers greater sensitivity for the detection of allergic disease than either test alone. Advances in allergy laboratory testing should improve the prognostic and diagnostic accuracy of food evaluations. Similar advances may improve the accuracy of aeroallergen diagnosis, which in turn may guide clinicians to prescribe more specific allergen immunotherapy Few researchers in our area have been published about laboratory testing for allergy, so we hope that our study will enrich the data base literature of the area with updated information through the large number of samples coming to KHMC being one of the major hospitals in the region.

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