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European Journal of Experimental Biology, 2012, 2 (6):2199-2205



Estimation of serum immunoglobulin E (IgE) level in allergic asthma and allergic rhinitis patients before and after treatment

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ABSTRACT

Estimation of serum immunoglobulin E (IgE) level is a valuable diagnostic parameter in Allergic Asthma and allergic rhinitis^[1,2]. This project work was done in collaboration with Dr. K. Sivanandhan who has been doing this research work for the last 20 years and has also established that Allergic Asthma and Allergic Rhinitis are curable diseases. Immunoglobulin 'E' (IgE) antibody has only been found in mammals and is associated with type I hypersensitivity reaction, where Immunoglobulin E level is found to be raised in a topic individuals and in some parasitic infestation like Schistosomiasis, Plasmodium falciparum etc... Atopic individuals (people who suffer from the Ig E mediated Allergies) can have upto 10 times the normal level of Ig E in their blood. In our study we took up 61 patients for IgE estimation both before and after treatment. The patients were treated by Dr. Sivanandhan with histaglobulin and other drugs. The Patients were from urban and rural areas comprising both male and females of all age groups. Out of 61 patients, 55 patients showed reduction in IgE Level (90.2%) and 6 patients showed no reduction in IgE level (9.8%) Clinically also, those patients with reduction in IgE level showed clinical improvement and these who did not have the reduction in IgE level also showed no clinical improvement. Among 61 patients 5 Patients showed 80% reduction in IgE level. 11 Patients showed 50- 80% reduction and 30 patients showed about 15-49% of reduction and 9 patients showed with 15% reduction IgE level after treatment. Hence, it is well established that the estimation of serum IgE level is a dependable laboratory data in patients suffering from allergic Asthma and allergic rhinitis.

Keywords: Atopic diseases, bronchial asthma, allergic rhinitis, serum Immunoglobulin E level, Histaglobulin therapy.

INTRODUCTION

Between 200 to 300 million people around the globe are suffering from asthma and the number is rising every year. India has an estimated 15 to 20 million asthmatics with a rough estimate of 10 to 15% prevalence rate among 5 to 11 year old children. Worldwide deaths from asthma have reached over 1,80,000 annually.

Allergy involves the production of a special class of antibody called immunoglobulin E. (IgE) which has only been found in mammals. These antibodies are bound to specific cells called mast cells, found in the skin, lungs and in many other tissues. Mast cells contain many powerful chemicals including histamine, that when released, causes an itching, itchy red rash through H1 receptor and other allergic manifestations such as watering of nose, sneezing, wheezing, cough etc. through H1 receptor. Estimation of Ig E may be an important one in treatments for allergy and asthma.

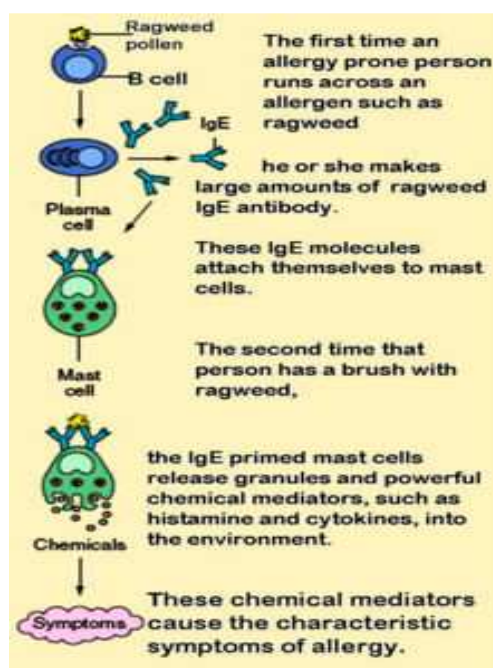
In biology IgE is a class of antibody that plays an important role in allergy and is especially associated with type I hypersensitivity. The serum level of IgE is 0.0003 mg/ml the lowest of all other types of antibodies. (IgG -13.5 mg/ml, IgA 3.5 mg/ml, IgM 1.5 mg/ml and IgD 0.03 mg/ml). The IgE is capable of triggering the most powerful immune reactions^[5].

IgE determination is valuable in the diagnostic assessment of patients with established or suspected allergic diseases^[4, 5]. Studies have shown that condition such as asthma, allergic rhinitis, eczema, urticaria, atopic dermatitis and some parasitic infections lead to increased IgE levels.

At present, allergic asthma and allergic rhinitis are considered as incurable diseases. But Dr. K. Sivanandhan at Pollachi has done the research work with histaglobulin + Drug therapy on allergy for 20 years and established that the above mentioned diseases are curable^[6]. We have associated with him with research work for the last 8 years.

In normal individuals the serum histamine binding capacity is 20 to 30 % whereas it is only 0 to 5% in allergic patients^[7]. After treatment the serum IgE level comes down and it is hypothesised that the serum histamine binding capacity of the patient rises with treatment. Hence serum IgE levels are estimated both before starting the treatment and 2 - 3 months after completion of the treatment.

We observed there was marked decrease in serum IgE level in those patients who showed clinical improvement with the histaglobulin along with medicines. Those patients who did not show fall in serum IgE level after treatment, did not show clinical improvement. Hence it is well established that the estimation of serum IgE Level is a dependable laboratory data in patient suffering from allergic asthma and rhinitis. The details of the study are presented below.



MATERIALS AND METHODS

Collection of sample

Blood sample was collected by vein puncture, allowed to clot and separate the serum by centrifugation at room temperature. If sera cannot be assayed immediately, they will be stored at 2-8⁰C for a week. Avoided repeated freezing and thawing of serum sample.

Principle of the assay

The MAGIWEL IgE (Merck) quantitative is a solid phase enzyme-linked immunosorbent assay (ELISA). The wells are coated with anti- IgE antibodies. The samples, standards and controls are incubated in the wells with enzyme conjugate which is another antibody directed toward a different region of IgE molecules and chemically conjugated with horseradish peroxidase^[8,9]. Unbound enzyme conjugate is washed off and the amount of bound peroxidase is

proportional to the concentration of the IgE present in the samples, standards and controls. Upon addition of the substrate and chromogen, the intensity of colour developed is proportional to the concentration of IgE in the serum.

Assay procedure and conditions

All the samples and reagents are kept in a room temperature (24-30°C), used new disposable tips for each specimen. Secure the desired number of coated wells in the holder. Dispensed 100uL of standards, controls or serum samples into appropriate wells, immediately added 100uL of UBI zero standard diluents into each well, then incubate for 30 minutes at room temperature. Removed incubation mixture and rinsed the wells 5 times with tap water(300uL)and the water thoroughly, dispensed 100uLof enzyme conjugate to each well and incubated 30 minutes; Removed the incubation mixture and rinsed with water five times. Dispense 100uL of solution A and then 100uL of solution B into each well and incubated for 10 minutes in room temperature. Added 50uL of stop solution and stopped the reaction and read O.D at 450 nm with a micro well reader

Washed the micro wells and remove water thoroughly. Pipetted all reagents and samples into the bottom of wells and avoided scratching the well. Vortex-mixing or shaking of wells is not required. Absorbance is a function of time and temperature of incubations. It is recommended to have reagents, samples and needed wells ready and assigned. It will ensure the equal elapsed time for each Pipetting without interruption. For the same reason, the size of the assay run each time should be limited. It is suggested to run no more than 20 patients with a set of reference standards in duplicate.

CALCULATION

Any microwell reader capable of determining at 450 nm may be used. The IgE value of patient is obtained as follows:

Plot the concentration (X) of reference standards against absorbance (Y) on full logarithmic paper.

Obtain the value of patient IgE by reference to the standard curve.

Expected value

Serum IgE may vary as result of season of that each its own serum IgE may vary as geographical location, diet and the year. It is recommended laboratory should establish expected normal range. Study of the expected concentration of IgE in a population of healthy non allergic individuals are complicated by the fact that some individual may have sub-clinical allergies, and have abnormal IgE concentration.

The geometric mean IgE values for healthy children have been reported to be age dependent and peak (28IU/mL) at the range of 10 years. For non atopic adults, the geometric mean IgE value was reported to be 14 IU/mL

TOTAL IgE

| Normal range | | | |
|--------------|-------|--------|-------|
| Age | Value | Age | Value |
| <1yr | <29.0 | 2-3yr | <45.0 |
| 1-2yr | <49.0 | 3-9yr | <52.0 |
| | | Adults | <87.0 |

Analytical sensitivity : 1.0IU/mL
Calibration range : upto 2000 IU/mL
Specimen require : 2mL random serum sample

RESULTS AND DISCUSSION

Serum IgE level was estimated both before and after treatment for 61 patients. Out of 61 patients 55 patients showed reduction in IgE level(90.2%). For 6 patients, the serum IgE level was not reduced. Accordingly the 6 (9.8%) patients did not showed clinical improvement.

The reduction in IgE level was 80% or above in 5 patients, 50-80% in 11 patients and 15-50% in 30 patients and less than 15% in 9 patients as indicated in figure 1.

Out of 61 cases 39 cases are from urban and 22 from rural area, So the occurrence is slightly higher in urban area (64%) when compared to rural area(36%) were tabulated in figure 2.

The females are affected more (32 patients-52%) when compared to male patients (29 patients-48%) figure 3.

The number of patients in the age group of 2-5 years is 2 (3.2%). The two patients are male (100%). In the age group of 2-5 years, 19 persons (31.2%). Among them male are 11 (57.9%) and female are 8 (42.1%). In the age group of 15 and above number of patients is 40 (65.6%), among which male are 16 (40%) female are 24 (60%) (figure 4).

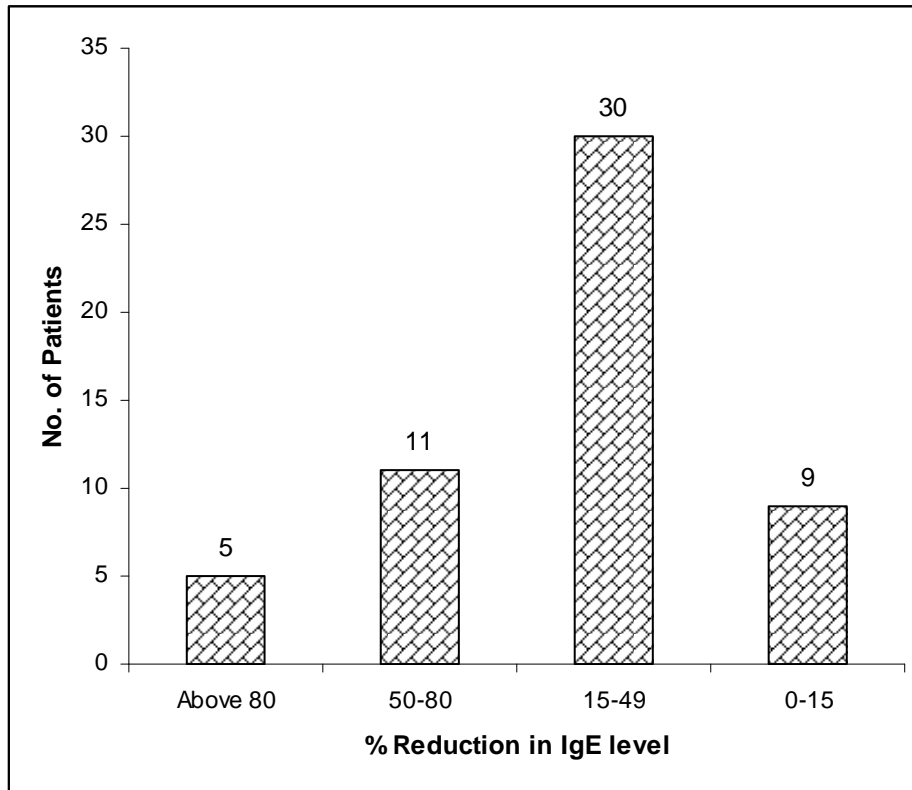


Fig. 1. Estimation IgE level in serum before and after treatment

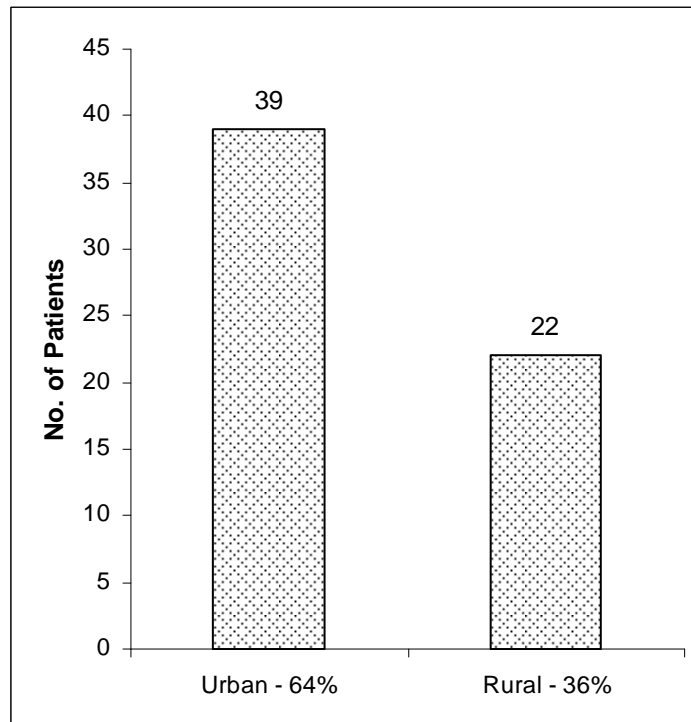


Fig. 2. Estimation IgE level in Rural and Urban Area

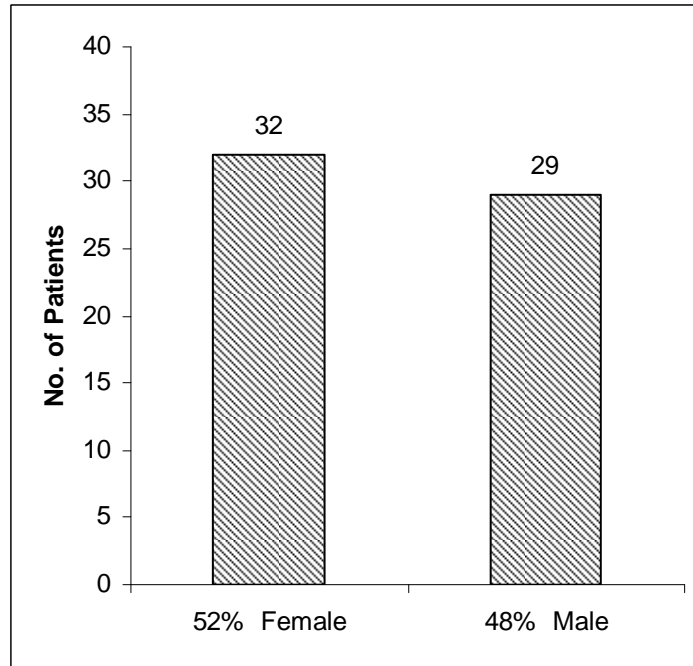


Fig. 3. Estimation IgE level among Female / Male

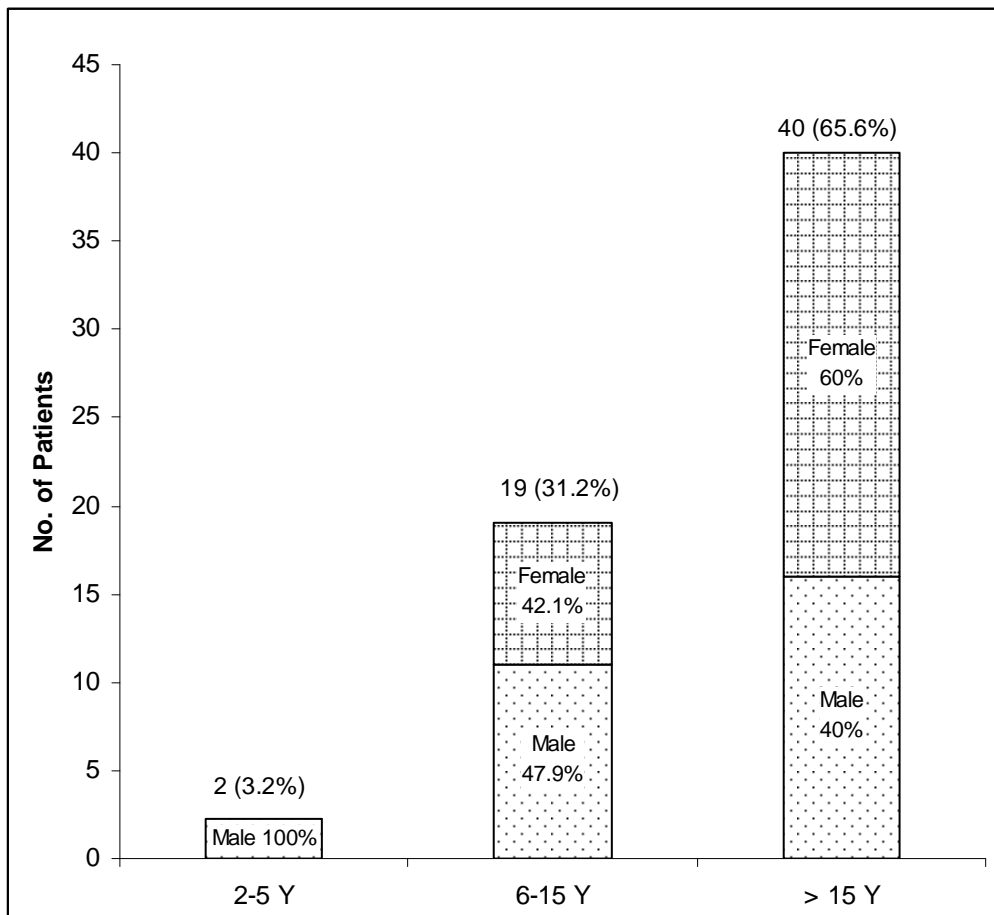
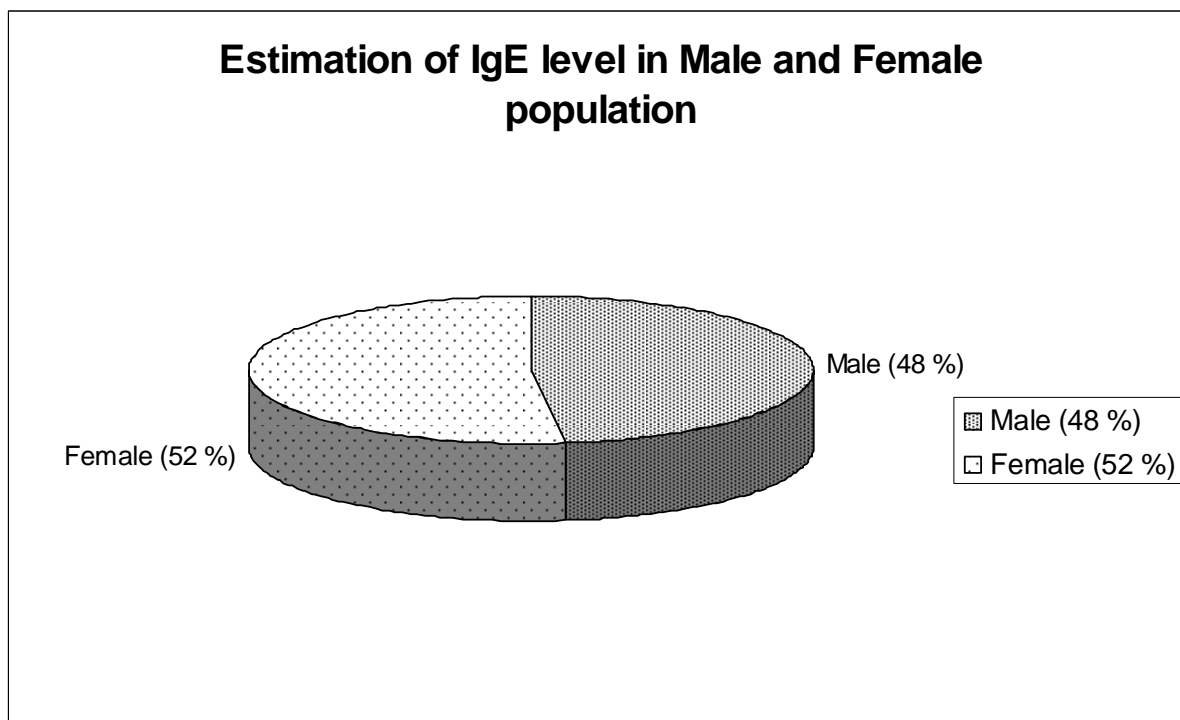


Fig. 4. IgE Age-wise out of 61 patients



DISCUSSION

At present allergic asthma and allergic rhinitis are considered as incurable diseases. But Sivanadhan (2007) has done the research work with histaglobulin therapy and established that the above mentioned diseases are curable. We have associated with him in the research work for the last 8 years. Between 100 and 150 million people around the globe are suffering from asthma and the number is rising. India has an estimated 15 to 20 million asthmatics and a rough estimate indicates a prevalence of 10 to 15% in 5 to 11 year old children. Worldwide deaths from asthma have reached over 1,80,000 annually.

In normal individual the serum histamine binding capacity is 20 to 30% whereas it is only 0 to 5% in allergic patients. Various researches had tried with histaglobulin introduced by parrot *et al* in various doses but without desirable results. In our study histaglobulin was given to asthma and allergic rhinitis patients after “preparing them” with antihistamines, mast cell stabilizers, bronchodilators etc.,

If the patients are properly prepared before starting histaglobulin therapy they responded well with the therapy. In the study a total number of 61 patients were involved which include patients of all age groups, both male and female and from urban and rural areas. The efficiency of the treatment was established by measuring serum IgE level both before and after treatment with histaglobulin along with clinical assessment of the patients.

We observed there was marked decreases in serum IgE level in those patients who showed clinical improvement with the histaglobulin along with therapy. Those patients who did not show fall in serum IgE level after treatment, did not show clinical improvement. So it is well established that the estimation of serum IgE level is a dependable laboratory data in patient suffering from allergic asthma and rhinitis.

CONCLUSION

Thus in this study comprising 61 patients having the complaints of allergic asthma with or with allergic rhinitis after histaglobulin therapy the serum IgE level definitely comes down. Hence estimation of serum IgE level is a dependable laboratory investigation both for diagnosing and establishing clinical improvement of allergic asthma with or without allergic rhinitis. Urban people are more affected than rural people due to allergic asthma. Adults are affected more than children. Females are slightly on higher ratio than males.

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