TO STUDY THE IMMUNE AND ALLERGENIC PROFILES OF ATOPIC ASTHMA PATIENTS

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CERTIFICATE

This is to certify that this dissertation entitled **TO STUDY THE IMMUNE AND ALLERGENIC PROFILES OF ATOPIC ASTHMA PATIENTS** is the bonafide original work of **Dr.N.JOTHIMURUGAN** in partial fulfillment of the requirement for MD (Branch -I) GENERAL MEDICINE examination of the Tamil Nadu Dr.MGR Medical University to be held in March 2008.

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DECLARATION

I, **Dr.N.JOTHI MURUGAN**, solemnly declare that this dissertation "**TO STUDY THE IMMUNE AND ALLERGENIC PROFILES OF ATOPIC ASTHMA PATIENTS"** is a bonafide record of work done by me in the Department of Medicine, Government Stanley Medical College and Hospital, Chennai under the guidance of **Prof.Dr.S.RAMASAMY, M.D.,** Addl. Prof. of Medicine, Government Stanley Medical College and Hospital, Chennai – 600 001.

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CONTENTS

INTRODUCTION

Nearly 300 million people in the world suffer from asthma[1] .Asthma is a common chronic inflammatory disease of the airways, with considerable heterogeneity both in its phenotype and in the underlying pathophysiology[2]. Both intrinsic or non-atopic and extrinsic or atopic cases of asthma are known. Extrinsic asthma is mainly a childhood disorder though the age of onset can vary; whereas intrinsic asthma is observed where the age of onset is above 45 yrs and is mainly due to the age induced changes in the lung function[3]. The majority of asthmatics (around 80%) are atopic, with manifestations of allergic diathesis including clinical allergy to aeroallergens and foods, or subclinical allergy apparent by skin test reactivity to allergen or elevated serum IgE. Approximately 7-10% of the Indian population, particularly children, is affected by atopic asthma [4, 5] .It has been estimated that 34% of the man days in India are lost due to airway related disorders, of which asthma is a major cause [6] .Asthma and atopy are considered complex traits, with evidence of both heritable and environmental factors contributing to their pathogenesis. Atopy is known to be a strong predisposing factor in the development of asthma[7]. Atopic asthma is a hereditary disease and is mediated both by genetic and environmental components. As its

pathogenesis is complex, management of the disease is also difficult. But prevention of the disease is possible if the allergic status of patients is known. However, due to the risk of anaphylaxis the allergy test is not being performed routinely in asthma clinics managing a lot of new as well as old patients. Due to this, poor asthma patients are not aware of these allergies and do not know the status of allergen positivity they have. In contrast, skin prick test is a test which carries less risk rate of anaphylaxis due to minimum possibility of allergen entry into systemic circulation [8] .

With this background, this study emphasizes the importance of the skin prick test in asthmatics and tries to correlate with the immune response in these patients. In addition, it tries to understand the prevalence of the allergen positivity in these patients and it also explores a few interesting Findings by pedigree analysis.

REVIEW OF LITERATURE

DEFINITION AND DESCRIPTION OF ASTHMA:

Asthma is a disorder defined by its clinical, physiological, and pathological characteristics. The predominant feature of the clinical history is episodic shortness of breath, particularly at night, often accompanied by cough. Due to complexity of its nature it is very difficult to define asthma. The following definition has been given by Global Initiative for Asthma [9]

"Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment".

It is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role, in particular, mast cells, eosinophils, T lymphocytes, macrophages, neutrophils, and epithelial

cells[10]. In susceptible individuals, this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning[10]. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment. The inflammation also causes an associated increase in the existing bronchial hyper responsiveness to a variety of stimuli.

ASTHMA DIAGNOSIS:

Asthma diagnosis is done on the basis of spirometry in individuals identified by certain key indicators outlined in the Clinical Practice Guidelines of Expert Panel Report-2, National Asthma Education and Prevention Program (2002) as shown in Box 1. Typically in spirometry, the maximal volume of air forcibly exhaled from the point of maximal inhalation (forced vital capacity, FVC) and the volume of air exhaled during the first second of the FVC (forced expiratory volume in 1 second, FEV1) is measured.

- **Airflow obstruction** is indicated by reduced FEV1 and FEV1/FVC values relative to reference or predicted values. A reduced ratio of FEV1/FVC (i.e., <65 percent) indicates obstruction to the flow of air from the lungs, whereas a

reduced FVC with a normal FEV1/FVC ratio suggests a restrictive pattern.

- Significant **reversibility** is indicated by an increase of ≥12 percent or 200 mL in FEV1 after inhaling a short-acting bronchodilator (American Thoracic Society 1991)

BOX 1. KEY INDICATORS FOR CONSIDERING A DIAGNOSIS OF ASTHMA

Consider asthma and performing spirometry if any of these indicators are present.* These indicators are not diagnostic by themselves, but the presence of multiple key indicators increases the probability of a diagnosis of asthma. Spirometry is needed to establish a diagnosis of asthma.

- Wheezing—high-pitched whistling sounds when breathing out—especially in children. (Lack of wheezing and a normal chest examination do not exclude asthma.)

- History of any of the following:
  - — Cough, worse particularly at night
  - — Recurrent wheeze
  - — Recurrent difficulty in breathing
  - — Recurrent chest tightness

- Reversible airflow limitation and diurnal variation as measured by using a peak flow meter, for example:

  — Peak expiratory flow (PEF) varies 20 percent or more from PEF measurement on arising in the morning (before taking an inhaled short-acting beta2-agonist) to PEF measurement in the early afternoon (after taking an inhaled short-acting beta2-agonist).

- Symptoms occur or worsen in the presence of:

  — Exercise

  — Viral infection

  — Animals with fur or feathers

  — House-dust mites
  (in mattresses, pillows, upholstered furniture, carpets)

  — Moulds

  — Smoke (tobacco, wood)

  — Pollen

  — Changes in weather

  — Strong emotional expression (laughing or crying hard)

  — Airborne chemicals or dusts

  — Menses

- Symptoms occur or worsen at night, awakening the patient.

*Eczema, hay fever, or a family history of asthma or atopic diseases are often associated with asthma, but they are not key indicators.

ROLE OF GENES AND ENVIRONMENT:

Heritability estimates of asthma vary between 36 and 79%. Allergen sensitization and subsequent exposure, dependent on both genetic and environmental factors, are widely understood to influence the development of atopic asthma. An association between total serum IgE level and asthma prevalence has been recognized in asthma patients of all ages [11, 12] .The linkage of total IgE levels to chromosome 5q highlights the importance of genetic factors in the development of sensitization[13, 14]. In addition, the level of environmental exposure to allergen is likely to be important because sensitization to different allergens in different patient populations has been associated with asthma[15, 16] and airway hyperresponsiveness[17]. The most prominent of these include *Alternaria* in children living in a desert environment[15] ,dust mite in adolescents living in central Virginia [16], and cat allergen in older men.

ALLERGY:

Without doubt, the casual correlation has been found between allergy and atopic asthma. These two phenotypes are components of atopy. Allergies affect around 30% of the adult population and 40% of children. The prevalence, severity and complexity of allergy in the population are rapidly rising [18]. Early diagnosis of allergy will be helpful

for primary prevention or at least for delaying the onset of asthma by avoiding allergens. The great majority of general practitioners have received little allergy teaching as students and no extra postgraduate training.

Allergy is a ‘hypersensitivity’ reaction, or exaggerated sensitivity, to substances which are normally tolerated. Such substances are known as allergens. Examples of common allergens include peanuts, milk, cats, horses, medicines and grass pollens. These allergens trigger the production of a harmful antibody, immunoglobulin E (IgE). In an allergic reaction, the interaction between the IgE and the allergen causes the release of inflammatory chemicals such as histamines and leukotrienes. These cause symptoms such as sneezing, itches, rashes and falls in blood pressure; they may also cause airway narrowing, which leads to shortness of breath and wheezing, and swelling which, if in the mouth, throat or airway, causes severe difficulty in breathing [19] .Sometimes symptoms are caused by other mechanisms, where IgE is not involved. These are often described as ‘intolerances’ to, for example, foods or medicines. Allergy practice deals with both IgE mediated and non-IgE-mediated reactions [20]. Allergic symptoms vary greatly. An individual may have a single symptom (for example, asthma) or multiple reactions (for example,

asthma, eczema and hay fever); swellings on the skin; or sickness [19]. The most extreme reaction of all is anaphylaxis.

INDOOR AND OUTDOOR ALLERGENS:

Though, both genetic and environmental factors are involved in the complex pathogenesis of asthma, genetic components are very complex due to the heterogeneity of every population. For example, one gene which is involved in asthma pathogenesis in a particular population, the same gene may not be significant in another population. These ethnicity variations are due to migration status of the population and many other factors[21]. Similarly environmental factors are also varies population to population. But it is predominantly determined by the current environment. There are some important environmental factors such as air pollution and some allergens. Among these, air pollution is having a major role in extrinsic asthma. In allergens, there are indoor and outdoor allergens. It has been shown by birth-cohort studies that sensitization to house dust mite allergens, cat dander, dog dander and aspergillus mold are independent risk factors. However, sensitization depends on the allergen, the dose, the time of exposure, the age, and probably genetics as well [22].

For some allergens, such as those derived from house dust mites and cockroaches, the prevalence of sensitization appears to be directly correlated with exposure [23]. So identification of the allergic status will be definitely helpful in preventing or at least delaying the onset of symptoms.

Common indoor allergens are following:

1. Dust mites and their faeces
2. Dander from pets and other animals having fur
3. Fungi
4. Cockroaches

PREVENTION OF ALLERGY:

Asthma has a significant effect not only on individuals but also on their families, and society also bears the brunt. Though there is no cure for asthma, appropriate management most often results in the achievement of control.

For this, there are few requirements:

1) Patient should know that to which allergens they are allergic. Some allergens such as those related to food allergy can be

recognized by the patients themselves but some invisible allergens may need help.

2) Awareness of avoiding the allergen to which they are allergic.

3) Encourage patients atopic asthma to do a family screening by which hidden or subclinical allergy can be traced out.

To evaluate this, this study has been performed to understand the level of awareness patients have and Skin Prick Tests (SPT) has been performed to their probands and blood relatives of them. Further, Immunoglobulin E measurement was performed not only for the proband but for relatives of the proband also.

Though pharmacologic interventions to treat established asthma are highly effective in controlling symptoms and improving quality of life, measures to prevent the development of asthma, and to avoid and reduce exposure to risk factors should be implemented wherever possible. This area is a focus of intensive research, but until such measures are developed prevention efforts must primarily focus on prevention of asthma symptoms and attacks. Due to combined genetic and environmental nature, it is theoretically difficult to prevent the onset of

asthma until a method is available to screen at the level of prenatal level and/or perinatal stages even before allergen sensitization. Though gene therapy has potential advantages, at the moment it is in an experimental stage due to the involvement of multiple known and unknown genes. Also, heterogeneity is very common among various populations which could restrict the generalized treatment modality even if it comes into widespread use.

Once allergen sensitization has occurred, it is difficult to prevent it.However, there are still a few possibilities to prevent or postpone the occurrence of asthma. Whether H1-antagonists (antihistamines) or allergen-specific immunotherapy can prevent the development of asthma in children who have other atopic diseases, still remains an area of investigation [(24).] But this approach is not validated experimentally. So, definitely this study will definitely give some clues which can act as tools for future possible fruitful prevention even in early childhood.

So, if we find environmental triggers, it will be helpful to control the symptoms or delay the onset of asthma. Triggers are referred to a variety of factors which cause asthma exacerbations. They are many factors including allergens, viral infections, pollutants, and drugs. Reduction of exposure to these triggers (avoiding foods/additives /drugs

known to cause symptoms) improves the control of asthma and reduces medication needs [25]. In the case of other factors (e.g., allergens, viral infections and pollutants), avoidance is best solution.

INDOOR ALLERGENS:

Among the wide variety of allergen sources in human dwellings are domestic mites, furred animals, cockroaches, and fungi.

ARTHROPODS:

DOMESTIC MITES:

Dust mites are not visible to naked eyes. They are eight legged arachnids and 0.3 mm long. Biologically they are related to ticks, spiders and scabies mites. They stay in the dust accumulating areas like beddings, carpets, fabrics and furnitures. Skin scales of human are the major source of food for house dust mites. Beddings are the major source of their infestation. There will be 10 to 1000 mites per gram of house dust. They require optimum conditions of temperature and humidity to survive and breed. They are mostly found in domestic houses rather than public places [26].

COCKROACH:

An allergy to cockroaches is similar to an allergy to dust mites. The cockroach allergens stem from their shed outer coverings (cuticles), their saliva, their eggs, and their feces. Cockroach allergens can be found in house dust and bedding. Avoidance measures for cockroaches include eliminating suitable environments (restricting havens by caulking and sealing cracks in the plasterwork and flooring, controlling dampness, and reducing the availability of food), restricting access (sealing entry sources such as around paperwork and doors), chemical control, and traps [(27).]

Various studies suggest that exposure to cockroach allergens is one of the most important risk factors for asthma in inner-city households. It was reported that asthma morbidity was highest in children with both a positive skin test response and a high exposure to the cockroach allergen in the bedroom by National Cooperative Inner-City Asthma Study [(27).] Further, it was also found that the most common sensitization was to cockroach allergen, with 47% of the subjects sensitized. Cockroach sensitization was also associated with a significant reduction in FEV_1 in this population in elder asthma patients with asthma in New York City[(28).] These evidences indicate that reducing exposure to cockroach allergens could be a valuable strategy to improve the health of inner-city residents.

MOTH:

In our environment, insects such as butterfly, moth, caddis fly, and chironomid were predominant and most abundant in the spring and autumn [29]. Antibody responses against silkworm wing and caddis fly wing were higher than those against silkworm body and caddis fly body respectively, indicating that the wing components, such as the microscopic scales and hair, are antigenic for asthmatic patients.

MOSQUITO:

Mosquito bites produce cutaneous reactions such as pruritic wheals and delayed papules. It also causes arthus-type local and systemic symptoms but anaphylactic reactions are very rare. Experimental evidence suggest that the various bite reactions result from sensitization to the mosquito saliva injected into the skin during feeding. Cutaneous sensitization to mosquito bites can be divided into five different stages ranging from the stages of immediate whealing and delayed bite papules, to the stage of non-reactivity. No desensitization treatment is generally available for mosquito allergy but it has recently been shown that cetrizine, a potent non-sedating antihistamine, is effective against the whealing and pruritus caused by mosquito bites [30].

POLLENS:

There are many type of pollens depending on the type of plant: grasses, weeds and trees. Pollen allergy is very is common in people with asthma. It may worsen asthma symptoms during the pollen seasons (usually in spring and early summer or during the dry season in tropical regions).It can cause outbreaks of asthma attacks after thunderstorms. It is usually caused by imported grasses, weeds and trees, which are wind pollinated. It is not usually caused by highly flowered plants as they produce less pollen (which is transported by bees) than wind pollinated plants. The importance of pollens causing pollinosis is well established and documented in India [31].

NEEM POLLEN:

Though neem tree has lot of medicinal properties its pollens act as allergens and allergy to the same has been reported. Neem trees are prevalent in south India and their pollination period is from April to June[31].

EUCALYPTUS POLLEN:

Eucalyptus is also a medicinal plant, the products of which have been shown to have various anti-inflammatory properties. In addition eucalyptus allergy has also been reported either in the form of pollen or in the form of contact dermatitis due to eucalyptus oil. Eucalyptus tree is

also prevalent in south India, it has two pollination periods per year; one is between February and March and the other is between July to October[31].

PARTHENIUM POLLEN:

Congress grass (Parthenium hysterophorus) is a common weed growing wild in the urban and semi urban environs of India [32]. Parthenium allergy is caused by direct and indirect contact with Parthenium hysterophorus which was introduced into India accidentally from the United States 20 years ago. Its distribution required optimum environmental conditions such as Indian climate. It causes severe contact dermatitis in a lot of people, which becomes chronic after longer exposure. During the dry season in India the mature plants crumble to a fine dust, which is scattered by the wind and becomes disseminated throughout the countryside. Permanent contact with this dust is the source of thes so called "airborne contact dermatitis" [33].

RICINUS COMMUNIS:

Ricinus communis (castor bean) is a plant species, Euphorbiaceae family, common in warm regions of the world. Though the allergenicity of its seed is well known, references are scarce regarding the role played by its pollen as a pneumo-allergen. Castor bean pollen is an allergen which causes respiratory (mainly nasal) symptoms [34].

MOULD ALLERGENS

It is prevalent in houses that are damp and have visible mould and can increase the risk of wheezing in some people. It can be avoided by removing visible mould by cleaning with bleach or other mould reduction cleaners, ensuring adequate natural ventilation, sealing leaks in bathrooms and roofs, removing indoor pot plants (which promote mould growth) and not working with garden compost or mulch. Although humidity promotes mould growth, dehumidifiers have been shown not to be helpful in asthma control. Reduction in fungal exposure can reasonably be expected to improve health. Removal of moisture from the indoors and proper maintenance of air filters can help in prevention and elimination of fungi from the home environment. Areas of present contamination can be cleaned with a dilute bleach solution, which kills viable colonies and removes their mycelia. If fungal contamination is not addressed early, substantial damage can occur, requiring professional remediation[35].

ASPERGILLUS FUMIGATUS:

Asthma has a significant impact on individuals, their families, and society. Although there is no cure for asthma, appropriate management that includes a partnership between the physician and the patient/family

most often results in the achievement of control. Fungal exposure has been associated with exacerbations from asthma and the number of fungal spores can best be reduced by removing or cleaning mold laden objects. In tropical and subtropical climates, fungi may grow on the walls of the house due to water seepage and humidity. To avoid this, the walls could be tiled or cleaned as necessary. Air conditioners and dehumidifiers may be used to reduce humidity to levels less than 50% and to filter large fungal spores. However, air conditioning and sealing of windows have also been associated with an increase in fungal and house dust mite allergens. Allergic bronchopulmonary aspergillosis (ABPA) is a complex disease, which is triggered by hypersensitivity for allergens of *Aspergillus fumigatus* [36]. .The clinical manifestations of the disease include remission and exacerbation, and also include the appearance of central bronchiectasis and progress to terminal stages with the presence of pulmonary fibrosis [37]

HOUSE DUST:

House dust is not a pure allergen but it is a mixture of many proteins predominantly biological contaminants such as house dust mite, spores of fungi and danders of pet. Complete avoidance of pet allergens is impossible, as the allergens are ubiquitous and can be found in many

environments outside the home, including schools, public transportation, and cat-free buildings. Although removal of such animals from the home is encouraged, even after permanent removal of the animal it can be many months before allergen levels decrease and the clinical effectiveness of this and other interventions remains unproven.

AIM AND OBJECTIVES

AIM:

- To study the immune and allergenic profiles of atopic asthma.

OBJECTIVES:

1. To study the allergen positivity predisposition in our environment.

2. To assess the impact of allergen positivity on IgE levels.

PATIENTS AND METHODS

RECRUITMENT OF THE STUDY POPULATION:

This study was a hospital based study, wherein, subjects were enrolled from the Allergy Clinic, Stanley Medical College, Chennai. Allergy clinic is the centre where most of the patients who complained of allergy related symptoms were referred from various other departments.

PERIOD OF STUDY:

January 2006 to January 2007 (thirteen months).

DESIGN OF STUDY:

Prospective study.

SELECTION OF SAMPLE:

Cases – ninety (90)

Controls-thirty six(35)

THE STEPS INVOLVED IN CARRYING OUT THE STUDY WERE:

1. Phenotyping and recruitment of patients.
2. Skin prick test for allergy
3. Allergy test grading

4. Serum isolation and IgE estimation

5. Statistical analysis

1. PHENOTYPING AND RECRUITMENT OF PATIENTS:

The probands were patients with asthma who visited the Allergy Clinic and fulfilled the following **inclusion criteria**:

1.Asthma diagnosed as per the definition of the American thoracic society ,1991 ie)

Significant **reversibility** is indicated by an increase of ≥12 percent or 200 mL in FEV1 after inhaling a short-acting bronchodilator (American Thoracic Society 1991).

2.A family history of asthma. (With either parent affected preferably first generation).

3.Age group were between 11-65 years.

4.Patients were recruited only after getting consent.

These probands were recruited based on the evaluation of clinical, family and environmental details using a standardized questionnaire (38). The questionnaire was designed to collect all the relevant information regarding the disease of the patient, and also the affection status of the

family members. Patients were clinically assessed and examined for a reported history of breathlessness, wheezing and allergic symptoms including conjunctivitis, rhinitis and dermatitis.

Exclusion criteria included:

1. Known chronic disease such as tuberculosis patients and symptoms suggestive of tuberculosis.
2. Age group of below 11 and above 65.
3. Patients who were not giving consent
4. Patients having negative family history.
5. Patients with history of smoking.
6. Known tropical pulmonary eosinophilic patients/other parasitic infections and symptoms suggestive of tropical pulmonary eosinophilia/other parasitic infections in the past three years.

In this study, patients were recruited from allergy clinic of Stanley medical college. Ethical approval was obtained from the ethical committee of Stanley Medical College(annexure A) . Written informed consent was taken from all individuals (parents in case of children)

participating in the study after explaining the objectives of the study (See Annexure B).

METHODOLOGY:

As a first step, screening phase was performed with short Questionnaire (see Annexure C) through which patients who were diagnosed as asthma were divided into two categories. First group being the patients who had a family history of asthma. Positive family history includes the history of wheezing and asthma related symptoms to the parents, grandparents or siblings. Patients who were having negative family history were excluded from further studies.

A detailed questionnaire was utilized to include the clinical history, economy, sanitation, the geographical region of origin and other important informations[38]. Only individuals with a family history of asthma/atopy were included in the study.

Spouses of probands were considered as healthy volunteers (referred to as normal controls) who answered negatively to a screening questionnaire for respiratory symptoms and on the basis of the criteria of having no symptoms or history of allergic diseases. Further, it was confirmed by allergy test for the spouses with their consent. Pulmonary function tests and skin prick test (SPT) were performed, wherever

consent was obtained. Individuals having a history of smoking and parasitic/helminthic infestations in the past three years were excluded from the study.

To determine awareness status of hereditary nature of asthma and to explore the hidden or sub clinical allergy status of the relatives, proband's relatives were recruited for both SPT and immune studies. Severity statuses of the probands were determined following the National Asthma Education and Prevention Program (Expert Panel Report-2, 2002) guidelines.

Based on the questionnaire and reported histories of alternative clinical symptoms and therapeutic interventions, patients with tuberculosis, parasitic infestations and chronic pulmonary diseases were excluded. The diagnosis of asthma was supported by pulmonary function test (PFT) in those co-operative with the testing and with supportive evidence in the form of high Absolute Eosinophils Count (AEC), total IgE etc.

At the time of enrollment, all the patients were subjected to a detailed clinical examination. In those who were co-operative with the testing, pulmonary function test (PFT) was carried out using a computer based spirometer.

Written informed consent was obtained from all parents for withdrawing blood samples from family members.

ASTHMA DIAGNOSIS AND CLINICAL PHENOTYPING

Probands who were diagnosed as asthma patient by clinical examination undervent clinical tests such as presence of BHR (defined as FEV_1/FVC below 80% at the time of attack and improvement by bronchodilator, skin prick test and total serum IgE associated with a positive response to at least one of the following questions depending upon the age of the patient (Have you ever had attacks of breathlessness at rest with wheezing (mainly in case of children)? Have you ever had an asthma attack at night? Have you ever got admitted to the hospital for asthma? Are you taking any asthma therapy?). Bronchial hyperresponsiveness (BHR) was measured in patients who had not taken medication for at least 12 hrs before the investigation.

2. SERUM ISOLATION AND IGE ESTIMATION:

BLOOD SAMPLE COLLECTION:

Eight-ten ml blood was obtained from individuals using vaccutainers (BD Biosciences, San Jose, CA, USA). For the sera separation, blood samples were collected in heparin coated vaccutainers

(BD Biosciences). Serum was separated by incubating whole blood obtained at room temperature (24-27^{o}C) for 2-3 hrs.

Total serum IgE was estimated as a quantitative measure of the atopicity of the patients and their parents. Whole blood obtained in a glass Vaccutainer (BD Biosciences) was incubated at room temperature (24 - 27°C) for 4 – 6 hours to separate the serum. Total serum IgE was estimated using a sandwich ELISA based assay procedure, as per manufacturer's instructions (Bethyl Laboratories, Montgomery, Texas, USA) and as used in a previous Indian study on asthma and atopy [(45).] Optical Density (OD) was measured at 490 nm and plotted against the standard concentration of h-IgE (SOFT-MaxPro, Molecular Devices, ELISA Reader, Minnesota, and USA). Data analysis was carried using the software Softmax Pro, Ver.2.0 (Molecular Devices, Minnesota, USA). Total serum IgE levels were expressed as International Units (IU). One IU is equivalent to 6.5ng/ ml of IgE. IgE values were represented as $\log_{10}$ -transformed data for analysis purposes. Total serum IgE was estimated using a sandwich ELISA based assay procedure, as per manufacturer's instructions (Bethyl Laboratories, Montgomery, Texas, USA) [(44).] Briefly, ELISA plates were coated with the capture antibody (1:100) at saturating levels by incubating at 37°C for 2 hrs. After three washes with TBS

(50mM Tris, 0.14M NaCl, 0.05% Tween-20), post coat solution (50mM Tris, 0.014M NaCl, 1% BSA) was added to the wells. After half an hour, the wells were washed three times with TBS and 100µl of the serum to be tested was added. Following this, the plates were incubated at 37^{o}C for 1 hr. After the incubation period, the plates were washed five times using TBS. 1:1000 dilution of the detection antibody (IgG-HRP conjugate) was added to the plates which were incubated at 37^{o}C for 1 hr. After 7 final washes with TBS, the color development was carried out using OPD substrate (40 mg/ml in Sodium Citrate Buffer, Sigma Aldrich, St Louis, USA). OD was measured at 490 nm and plotted against the standard concentration of h-IgE (SOFT-MaxPro, Molecular Devices, ELISA Reader, Minnesota, USA). Data analysis was carried out using the Softmax Pro, Ver2.0, software (Molecular Devices, Minnesota, USA). Total serum IgE levels were expressed as International Units/ml (IU/ml) of the serum [39]. One international unit is equivalent to 6.5 ng/ml of IgE. The IgE values were $\log_{10}$ transformed for any further analyses.

3. SKIN PRICK TEST:

Skin prick test is easy to perform and results are obtained immediately it is also cost effective. It can be performed by any

practitioner who has training in the technique and in the interpretation of results.

The test is based on the introduction of purified allergens into the dermis resulting in an IgE-mediated response, which is characterized by an immediate wheal and flare reaction. When the allergen is introduced into the skin on a previously sensitized individual, IgE molecules on the surface of a mast cell are bridged and degranulation of the mast cell occurs. Pre-formed granules containing histamine are released followed by progressive infiltration of the dermis by eosinophils and neutrophils which have been attracted to the site by chemotactic factors.

SPT can be performed on the volar or inner aspect of the forearms avoiding the flexures and the wrist areas. The procedure should be explained to the patients. The skin should be cleaned with spirit or 70% ethanol. A grid is marked with a pen at 2 cm intervals and a drop of the relevant allergen placed on the arm at the end of each line. The pattern should follow corresponding list of allergens used for easy identification.

A lancet with 1 mm point should be used to prick the skin through the drop. With the so-called "prick through drop" method it is unnecessary to scratch or lift the skin and no blood should be drawn. The

needle should be wiped with dry gauze between each prick, in order to prevent carry-over of allergens.

The allergen should be blotted off the test site. Reactions should occur within 10-15 minutes after which the results can be assessed. A positive and negative control must be included in each series of tests. The negative control solution is the diluent used to preserve the allergen extract. The positive control solution is a 1 mg/ml histamine hydrochloride solution.

Several types of allergens such as indoor, outdoor, food, mould allergens were selected and used for the skin prick test with both negative and positive controls. They were House dust mite, Azadirachta indica, Eucalyptus, *Parthenium hysterophorus,* Moong dal, *Alternaria tenuis Ricinus communis,* cocus nucifera, *Albizzia lebbeck*, Cockroach, *Aspergillus fumigates,* Mosquito, Moth, and Grain dust rice. Allergenic extracts in 50% glycerinated buffer were used for the test [40]. Histaminic acid phosphate (1mg/ml) and 50% glycerinated buffer was used as positive and negative controls, respectively. Allergens, positive and negative controls were purchased from *ALL CURE PHARMACEUTICALS, DELHI, INDIA.*

4. GRADING OF ALLERGY [41-44]:

As described previously [41-44], SPT was done and was graded. A reaction of 3 mm greater than the negative control is regarded as positive.

Grading may be expressed as a percentage of the positive histamine control or may be measured as follows:

+	No wheal, 3 mm flare
++	2-3 mm wheal with flare
+++	3-5 mm wheal with flare
++++	>5 mm wheal, may have pseudopodia

Clinical data on the diagnosis of asthma and atopic diseases or other respiratory disorders, their duration, skin problems, types and doses of medications, and history of tobacco smoking were obtained by filling up a detailed questionnaire. Details of economic status, environmental factors, and geographical region of origin were also noted. Individuals having history of active smoking in the past three years and/or suffering from parasitic or helminthic infestation were excluded from the study. The affection status was also noted for all the family members recruited in the study.

STATISTICA ANALYSIS: Data are expressed as mean ± SEM. This was done with JMP Statistical software. Significant differences between two groups were estimated using unpaired Student *t*-test. Statistical significance was set at $p < \text{or} = 0.05$.

ETHICAL COMMITTEE APPROVAL:

The study was submitted for the approval of the ethical committee

meeting held at Govt. Stanley Hospital and approval was obtained (ANNEXURE A).

OBSERVATIONS AND RESULTS

IgE VALUES IN PATIENTS AND CONTROLS

As mentioned in methods IgE was measured in sera and it was compared between patients and controls.

TABLE:1

Groups	***IgE in IU/ml***	***SIGNIFICANCE***
Patients (n= 90)	236.8 ± 28.3	P=0.0642 NOT SIGNIFICANT
Controls (n=35)	153.7 ± 27.6	

p VALUE=0.0642.Patients show higher IgE than controls though it is not statistically significant.

GENDER DIFFERENCE IN IGE LEVELS:

TABLE 2:

S.NO.	**SEX**	**IgE in IU/ml**	**SIGNIFICANCE**
1.	FEMALES(n=55)	206 ± 32.716	P=0.1713 NOT SIGNIFICANT
2.	MALES(n=35)	287 ± 51.824	

Here it is seen that males have higher IgE values than females however p value is 0.1713 is nil significant.

ECONOMIC STATUS AND IgE LEVELS;

TABLE 3:

ECONOMY	TOTAL NO.	IgE in IU/mL	SIGNIFICANCE
<1500	n=64	285 ± 39.532	P=0.0699 NOT SIGNIFICANT
1500-3000	n=26	160 ± 31.028	
>3000	-	-	

It shows that poor socioeconomic status people have higher IgE values as the p value is 0.0699.

MODE OF LIVING AND IgE LEVELS:

TABLE 4:

MODE OF LIVING	TOTAL NO.	IgE in IU/mL	SIGNIFICANCE
RURAL	n=26	267 ± 57	P=0.7247 NOT SIGNIFICANT
URBAN	n=52	244 ± 36	

p value shows 0.7247.

No correlation between IgE and mode of living was found.

FREQUENCY OF ALLERGEN POSITIVITY:

TABLE 5:

S.No.	***Allergens***	***Negative***	***Positive***
1	House dust mite	57	31
2	Amaranthus spinosus	75	13
3	Brassica campestris	78	10
4	Azadirachta indica	81	6
5	Parthenium hysterophorus	73	15
6	Prosopis juliflora	78	9
7	Ricinus communis	81	7
8	Adhatoda vasica	75	12
9	Aspergilus fumigatus	76	12
10	Cockroach Male	36	51
11	Moong Dal	77	11
12	Mosquitoes	77	9
13	Moth	32	56
14	Grain dust rice	30	58
15	Eucalyptus tereticornis	58	30
16	House dust	67	21
17	Albizzia lebbeck	76	10

This data clearly shows that cockroach, moth and grain dust rice have shown more positivity than other allergens.

Prosopis juliflora, ricinus communis, adhatoda vasica, aspergillus fumigatus are less allergenic.

GENDER DIFFERENCE IN ALLERGEN POSITIVITY:

The difference was calculated after normalization by gender ratio. (Difference between allergy positivity was divided by gender ratio). As shown in Table 6, females have more tendency for allergen positivity.

Table 6 – Female/Male ratio for SPT positivity:

Allergen	Female/Male
House dust mite	1.7
Amaranthus spinosus	1.3
Brassica campestris	1.3
Azadirachta indica	3.5
Parthenium hysterophorus	2.3
Prosopis juliflora	0.9
Ricinus communis	0.4
Adhatoda vasica	1.7
Aspergilus fumigatus	1.7
Cockroach Male	1.0
Moong Dal	1.0
Mosquitoes	0.7
Moth	0.9
Grain dust rice	0.9
Eucalyptus tereticornis	0.9
House dust	0.9
Albizzia lebbeck	0.8

Females and males showed equal rate of allergy positivity for most of the allergens. However, females have shown the predominant positivity for House dust mite, Amaranthus spinosus, Brassica campestris, Azadirachta indica, Parthenium hysterophorus, Adhatoda vasica, and Aspergilus fumigatus.

ALLERGY POSITIVITY AND IGE LEVELS:

Table 7. Comparison of Total Ig E levels with allergen positivity:

Allergens	SPT Negative	SPT Positive	P value
House dust mite	253.837±40.848	206.538±30.362	0.4256
Amaranthus spinosus	248.291±32.661	172.187±34.588	0.3385
Brassica campestris	247.955±31.478	151.916±37.728	0.2794
Azadirachta indica	228.768±29.309	237.657±74.029	0.935
Parthenium hysterophorus	252.158±33.196	164.031±36.796	0.2397
Prosopis juliflora	232.315±30.234	296.261± 90.74	0.5171
Ricinus communis	232.111±28.94	312.546±141.08	0.5093
Adhatoda vasica	240.074±1.359	216.518±63.923	0.775
Aspergilus fumigatus	234.288±29.734	252.197±90.381	0.828
Cockroach Male	256.492±42.608	224.433±38.783	0.5829
Moong Dal	244.148±31.911	186.598±39.778	0.5004
Mosquitoes	240.185±31.768	201.036±54.475	0.678
Moth	156.711±33.769	284.24 ± 39.207	**0.0286**
Grain dust rice	232.787±45.766	238.823±36.083	0.9204
Eucalyptus tereticornis	188.995±33.342	326 ±48.739	**0.0202**
House dust	232.739±33.034	250.146±55.522	0.7968
Albizzia lebbeck	213.94 ± 26.34	318.215±110.25	0.2079

This table shows p value of 0.0286 for moth and p value of 0.0202 for eucalyptus.both are statistically significant.

SKIN PRICK TEST GRADING AND IGE LEVELS:

HOUSE DUST MITE:

TABLE 8:

S.NO.	LEVELS	IgE IN IU/mL	SIGNIFICANCE
1.	1+	435 ± 166	P=0.2693 NOT SIGNIFICANT
2.	2+	122 ± 30.13	
3.	3+	328 ± 111.43	
4.	4+	406 ± 76.91	

p value =0.2693 .Though 4+ shows higher IgE ,it is not statistically significant.

AMARANTHUS SPINOSUS:

TABLE 9:

S.NO.	LEVELS	IgE IN IU/mL	SIGNIFICANCE
1.	1+	145 ± 53.87	P=0.2214 NOT SIGNIFICANT
2.	2+	362 ± 158.08	

Higher IgE for 2+ skin prick test grading though it is not statistically Significant.

BRASSICA CAMPESTRIS:

TABLE 10:

S.NO.	LEVELS	IgE IN IU/mL	SIGNIFICANCE
1.	1+	131 ± 39.74	P=0.0543 SIGNIFICANT
2.	2+	524 ± 297.81	

p value =0.0543 .significant difference is there for this allergen.

AZADIRACHTA INDICA:

TABLE 11:

S.NO.	LEVELS	IgE IN IU/mL	SIGNIFICANCE
1.	1+	272 ± 79.77	P=0.0432 SIGNIFICANT
2.	2+	584 ± 522.82	
3.	3+	865	

p value =0.0432 shows significant correlation between skin prick test grading and IgE values.

PARTHENIUM HYSTEROPHORUS:

TABLE 12:

S.NO.	LEVELS	IgE IN IU/mL	SIGNIFICANCE
1.	1+	172 ± 42.18	P=0.0476 SIGNIFICANT
2.	2+	442 ± 332.54	

p value=0.0476 .It is statistically significant..

PROSOPIS JULIFLORA:

TABLE 13:

S.NO.	LEVELS	IgE IN IU/mL	SIGNIFICANCE
1.	1+	300 ± 89.29	P=0.3288 SIGNIFICANT
2.	2+	588 ± 518.4	

Here the p value =0.3288.higher levels of skin prick test Shows higher IgE values though they are not Statistically significant.

TABLE 14:

COCKROACH:

S.NO.	LEVELS	IgE IN IU/mL	SIGNIFICANCE
1.	1+	336 ± 111.25	P=0.9082 NOT SIGNIFICANT
2.	2+	288 ± 77.5	
3.	3+	288 ± 107.43	

p value =0.9082.it's not significant statistically.

MOONG DAL:

TABLE 15:

S.NO.	LEVELS	IgE IN IU/mL	SIGNIFICANCE
1.	1+	402 ± 194.67	P=0.9874 NOT SIGNIFICANT
2.	2+	171 ± 70.41	

Skin prick test grading for moong dal and IgE values are not Statistically correlated.

MOSQUITO:

TABLE 16:

S.NO.	LEVELS	IgE IN IU/mL	SIGNIFICANCE
1.	1+	406 ± 195.43	P=0.7106 NOT SIGNIFICANT
2.	2+	240 ± 95.65	

p value =0.7106 .No correlation was found statistically.

MOTH:

TABLE 17:

S.NO.	LEVELS	IgE IN IU/mL	SIGNIFICANCE
1.	1+	369 ± 77.306	P=0.6123 NOT SIGNIFICANT
2.	2+	335 ± 81.645	
3.	3+	260 ± 98.816	
4.	4+	105 ± 43.012	

p value=0.6123

It shows inverse correlation with IgE values.

GRAIN DUST RICE:

TABLE 18:

S.NO.	LEVELS	IgE IN IU/mL	SIGNIFICANCE
1.	1+	332 ± 111.94	P=0.8293 NOT SIGNIFICANT
2.	2+	256 ± 55.04	
3.	3+	276 ± 134.39	

p value=0.8293.Not significant statistically.

HOUSE DUST:

TABLE 19:

S.NO.	LEVELS	IgE IN IU/mL	SIGNIFICANCE
1.	1+	398 ± 111.94	P=0.318 NOT SIGNIFICANT
2.	2+	173 ± 105.62	

p value =0.318.No significance was found statistically.

ALBIZZIA LEBBECK:

TABLE 20:

S.NO.	LEVELS	IgE IN IU/mL	SIGNIFICANCE
1.	**1+**	**614 ± 259.39**	P=0.2314 NOT SIGNIFICANT
2.	**2+**	**258 ± 134.36**	

p=0.2314.It is not significant statistically.

DISCUSSION

IGE IN PATIENTS AND CONTROLS:

The range of age for Patients and controls were 11 to 65 years. So as shown in Table I, the IgE levels of patients were higher than normal control individuals though it is not statistically significant.

GENDER DIFFERENCE IN IGE LEVELS:

Our study shows that males have higher IgE values as compared to females The p value is 0.1713, which is not significant statistically.

This is really interesting and controversial because many atopic symptoms are having maternal inheritance. For example, recently a study has shown that mitochondrial haplotype is associated with IgE levels[45]. Consequently gender variation in IgE levels could be due to the following mechanisms: 1) Genetic 2) Hormonal and 3) Environmental[46]. At the time of birth, genetic factors have a main role in determining a higher IgE levels in male rather than female infants [47]. In adults, a higher exposure to environmental factors in males as compared to females could have a role in causing higher IgE levels in males [48]. This is true not only for antigens found in the outdoor environment but also for antigens found in

the indoor environment like dust mite and cats, with males having higher specific IgE antibodies for these antigens as well. [49].

ECONOMIC STATUS AND IGE LEVELS:

Our study also shows that people frpm the lower socioeconomic strata have higher IgE levels ,though not significant statistically.

There was an inverse correlation between IgE levels and economic status. . However this was not found to be statistically significant. The reasons for this are not clear, but it could be due to effective disease management in the higher socioeconomic groups in contrast with an irregular treatment and a lack of awareness in lower socioeconomic groups. To illustrate this point it was observed during the course of this study that poorer patients attended the allergy clinic for treatment only after a protracted period after the onset of an acute attack, did not avail of any emergency treatment.

MODE OF HOUSING AND IGE VALUES:

Our study shows no correlation between IgE and the mode of housing.

This is really surprising because there is much evidence to suggest that pollution and contamination can act as triggering factors for the development of an asthma attack [50]. This could be explained by the Hygiene hypothesis. The hygiene hypothesis considers that there is an inverse association between family size and manifestations of atopy in early life to childhood. [51, 52] .Along with these changing patterns of microbial exposure, the decline in naturally occurring infectious diseases and some vaccinations (eg, against tuberculosis), form the background for this hypothesis

Exposure to microbes, either in the form of active infection or as a sub clinical infection may initiate protective responses[53]. The derivatives of microbes, which are primarily recognized by the innate immune system, may drive protective responses, especially at the cytokine level. This exposure may play a critical role in the shaping of the immune response when encountered at important stages during the maturation of immune responses. This could result in the development of immune tolerance to potential allergens. A major basis for the hypothesis is that improved hygienic conditions in Western or developed countries results in less infection-driven or microbial pressure during early but critical time periods in early childhood. But this kind of observation had not been

found in our population. However this needs well regulated study design. Th1 responses are predominantly mediated by interferon (IFN)-gamma and interleukin (IL)-12 productions, whereas Th2 responses are primarily associated with IL-4, IL-5, IL-13 (and IL-10) production [(54).] In association with reductions or altered exposures to infectious agents or their components, it is proposed that Th2 immunity, predominating from birth, dominates through critical childhood periods, resulting in the higher incidence of atopy and asthma. Various studies have advanced the theory that fecal contamination of the environment (and possibly infections such as hepatitis A), and unhygienic food handling may similarly protect against development of atopy. However, results from this study are not sufficient enough to support the hypothesis.

FREQUENCY OF ALLERGEN POSITIVITY:

Our study shows a higher rate of allergen positivity with cockroach, moth and grain dust rice as compared to other allergens.

In this study, no signs of anaphylaxis was found while performing SPT. As shown in the Figure, cockroach, moth, and grain dust rice showed high incidence of allergy positivity. This indicates that outdoor allergens such as pollens have only a smaller role in causing asthmatic attacks in our population. Rather indoor allergens such as cockroach

have a predominant role in determining the prevalence of allergic symptoms. The cockroach allergy was first recognized in 1960s and identified as an underlying cause of asthma morbidity [55].

Moreover these results suggest that moth allergy is also a factor of particular importance in causing allergy-induced bronchial asthma in our patients. As moths are attracted readily by artificial lights and often fly into houses, these insects are especially suspect as important factors in extrinsic asthma [56].

Very importantly, grain dust rice allergy also is of particular importance in our population, as rice is the staple cereal of this region with rice being cleaned indoors in many households. Furthermore it is seen that the prevalence of this allergy is almost equal in males as well as in females as shown in the Figure. This indicates that the allergen may spread throughout the house during the process of rice cleaning which is done before cooking rice.

In addition to these major allergens, house dust mite, eucalyptus tereticornis and house dust are the other important antigens showing allergen positivity. Among these, house dust mite allergy is unique in that it is a universal health problem. House dust mite allergy has been reported in 1920s by Cooke and Kern and later it was found that mites are

involved predominantly in the development of dust allergy [57, 58]. Later it was observed that mites are the major source of allergens for an asthma and allergic rhinitis. The two most important species are *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae.* Since mites live and thrive in many sites throughout the house, they are difficult to reduce and impossible to eradicate. No single measure is likely to reduce exposure to mite allergens, and single chemical and physical methods aimed at reducing mite allergens are not effective in reducing asthma symptoms in adults. An integrated approach including barrier methods, dust removal, and reduction of microhabitats favorable to mites has been suggested, although its efficacy at reducing symptoms has only been confirmed in deprived populations with a specific environmental exposure.

Biological agents have an important role in causing indoor air pollution. The indoor air environment has changed with the introduction of soft furniture's, carpets etc. There is an increased indoor relative humidity and decreased ventilation due to these modernizations. However due to these changes airborne allergens have increased significantly. Indoor air is the mixture of physical, chemical and biological contamination.

The major biological contaminants are dust mites and their feces, dander from animals and molds. In this study it is found that predominant allergens are indoor allergens rather than pollens which are outdoor allergens. So in contrast to our belief, it is very much essential to control indoor allergens rather than focus on outdoor allergens in our population. The relationship between human health and indoor air environment is an area of growing interest. It needs further detailed investigations.

GENDER DIFFERENCE IN ALLERGEN POSITIVITY:

Females and males showed equal rate of allergy positivity for most of the allergens. However, females have shown the predominant positivity for House dust mite, Amaranthus spinosus, Brassica campestris, Azadirachta indica, Parthenium hysterophorus, Adhatoda vasica, and Aspergilus fumigatus.

This predominance was very common in various pollens and house dust mite. House dust mite allergy predominance can be explained by increased staying time in indoor environment. In contrast, it was not true for other indoor allergens. But the predominance of pollen allergens in females is really very interesting. This needs further investigations.

ALLERGY POSITIVITY AND IGE LEVELS:

To determine the participation in allergy in the production of total IgE levels, the comparison was done between allergy positivity and total IgE levels. Only Moth and eucalyptus allergy have shown the significant correlation between the allergy positivity and total IgE levels. The reasons for this are not clear at the moment.

SPT GRADING AND IGE LEVELS:

HOUSE DUST MITE:

Higher IgE levels were found with lower grading. However the levels were not correlated with serial grading of SPT positivity. This indicates that quantity of allergens entering into the body is not equivalent to the production of IgE levels. The reason for controversial relation between allergy grading and IgE levels is not clear. Similar controversial reports have been found in the dose response relationship between symptoms and dust mite allergen exposure [59]. Marks et al have found that the concentration of mite allergens is not higher in the houses of asthmatic patients with mite allergy in the other houses [60].

AMARANTHUS SPINOSUS:

There was a good correlation between IgE levels and SPT positivity, that is lower IgE levels were correlated with lower allergy grading and vice versa .

BRASSICA CAMPESTRIS:

Almost similar correlation which was found with Amaranthus spinosus was observed with this allergen also.

AZADIRACHTA INDICA:

Surprisingly, the similar trend which was observed in brassica campestris pollen was found with this allergen.

PARTHENIUM HYSTEROPHORUS AND PROSOPIS JULIFLORA:

It is very interesting that there is a good correlation between allergy grading and IgE levels in case of pollens. This dose relationship is not found in literature, undoubtedly it might give various interesting findings if we explore in detail.

COCKROACH:

Interestingly there was no correlation was found between allergy grading and IgE levels. A similar relation was observed in house mite allergy as was found with cockroach allergy.

MOONG DAL:

There was an opposite correlation found between in allergy grading and IgE levels in case of this food allergy. The reasons are not known. It could be due to tolerance mechanisms. Because this kind of mucosal tolerance has been found in case of mice model of asthma when chicken egg ovalbumin has been given orally[61]

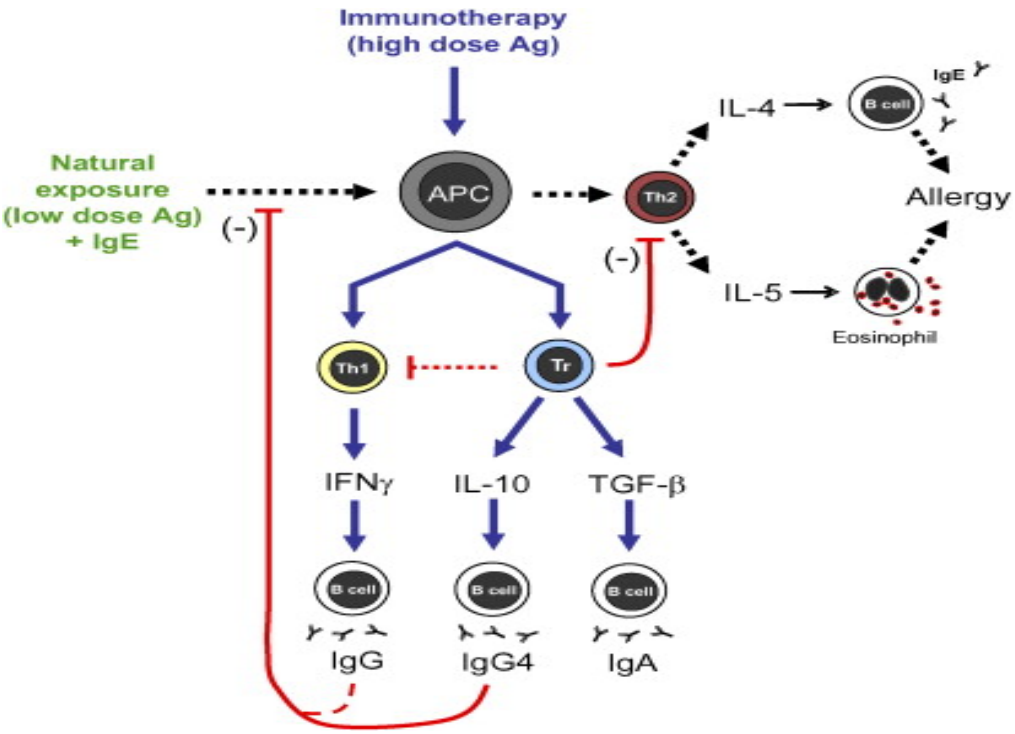
MOSQUITO AND MOTH:

Surprisingly, a similar observation which was found with mosquito allergy was observed in case of moth allergy. It is very interesting that controversial relationship or inverse correlation has been observed in case of in case of insect allergies such as house dust mite, mosquito and moth. This could be due to the following reasons:

1) Tolerance

2) Endogenous digesting mechanisms such as chitinase.

IMMUNOTOLERANCE:



As shown in the above Figure, high dose allergens are in fact act as a therapeutic modality [62]. When the allergens enter into our body mostly through mucosal surfaces, innate mucosal immune system, represented by epithelial and other mucosal cells and their products, is able to recognize them by pattern recognition receptors such as Toll-like receptors, CD14 and others. These local and systemic immune responses are later replaced by inhibition, by induction of mucosal (oral) tolerance. Characteristic players of mucosal immunity are: well developed innate defense mechanisms, characteristic populations of unique types of lymphocytes, colonization of the mucosal and exocrine glands by cells originating from

the mucosal organized tissues ('common mucosal system') and preferential induction of inhibition of the responses to non dangerous antigens (mucosal tolerance). Various chronic diseases, including allergy, may occur as a result of genetically or environmentally induced changes in mechanisms regulating mucosal immunity and tolerance. These mechanisms lead to cause the impairment of mucosal barrier function, disturbed exclusion and increased penetration of microbial, food or airborne antigens into the circulation and further lead to exaggerated and generalized immune responses to allergens [63, 64].

Recently, a mammalian homologue of an enzyme associated with parasitic infection in lower organisms, including amphibians and insects[65] and [66], has been shown to be expressed in Th2-mediated inflammation such as asthma [67]. Acidic mammalian chitinase (AMCase) is a 50-kDa protein that contains a 30-kDa N-terminal catalytic domain that can hydrolyse chitin, a polymer of *N*-acetylglucosamine [68]. Chitin is not expressed in mammalian systems but is abundant in the structural coatings of fungi [69], the exoskeleton of many arthropods [70] and parasitic nematodes [71]. AMCase is a member of the glycosyl hydrolase 18 family (EC 3.2.1.14).

GRAIN DUST RICE , HOUSE DUST AND ALBIZZIA LEBBECK:

All these allergens do not show any significance with skin prick test grading and ige values.The opposite correlation has been found with house dust allergy. This could be due to the fact that house dust is the mixture of many biological mixtures including dust mite allergens.

FAMILY ANALYSIS:

Many patients are unaware of the reasons for frequent sneezing. But if the allergy had been diagnosed earlier, it could be controlled by avoiding those allergens. For this reason, the relatives of the patients were recruited for the study. The non-blood related individuals such as spouses of the probands were taken as normal individuals for the comparison of IgE levels. Blood related individuals were recruited for the diagnosis of allergy irrespective of whether they had allergy related symptoms or not. Our preliminary analysis has shown that 20% of the individuals who had mild allergic symptoms showed positive allergy for at least one allergens. 10 % of the individuals who did not have any allergy related symptoms showed positive allergy to at least one allergen. As atopy is a hereditary disease, it seems that allergy checkup for at least a common allergen for relatives of the allergic patient could reveal unknown or hidden allergies.

SUMMARY

The present study is an attempt to understand the immune and allergenic profiles of atopic asthma patients.

The sample in this study consisted of 90 patients, who visited the allergy clinic in Stanley Medical College hospital for a period of twelve months.

This study concentrates mainly on the importance of skin prick test in bronchial asthma patients. This test reveals the allergy status of the individual,and this knowledge of the offending allergens can be used to avoid them.

Our study reveals lesser IgE in female patients compared to male patients and IgE levels are very high in low socio-economic groups compared to high socioeconomic groups.

The incidence of allergenicity is more common in females when compared to males.Moth and eucalyptous allergens show significant association between allergy positivity and IgE levels.

Allergens like brassica campestris, Azadirachta indica,parthenium hysterophorus shows higher IgE values with higher skin prick test grading, which is statistically significant.

CONCLUSION

1. The allergic status of the individual can be assessed with the help of skin prick test. This information can be used for avoidance of allergens.

2. Female patients have lesser IgE compared to male patients

3. There is an inverse correlation between IgE levels and socio economic status.

4. Cockroach, Moth, and Grain dust rice are shown the high allergy positivity while azadirachta indica, prosopis juliflora, ricinus communis and mosquitoes are shown the lower allergy positivity.

5. Females have more tendency for allergen positivity. This predominance was very common with respect to various pollens and house dust mite.

6. There was a significant association between allergy positivity and IgE levels in case of moth allergy and eucalyptus allergy.

7. There was an inverse correlation between allergy severity grading and IgE levels in case of insect allergy and direct correlation with pollen allergy.

LIMITATIONS AND SUGGESTIONS

1. Skin prick test is a safe procedure, which can be performed in any allergy clinic with minimum precautions due to the lesser chance of anaphylaxis.

2. SPT test could help in revealing hidden allergies.

3. Ideally the allergen grading has to be compared with allergen specific IgE. But for this, we need a purified powdered allergen.

4. The results are better if we have ruled out the other conditions which increase the serum IgE levels, like other parasitic /helminthic infections.

5. The study will be better if we might have compared pulmonary function test with IgE levels and skin prick test to assess the severity.

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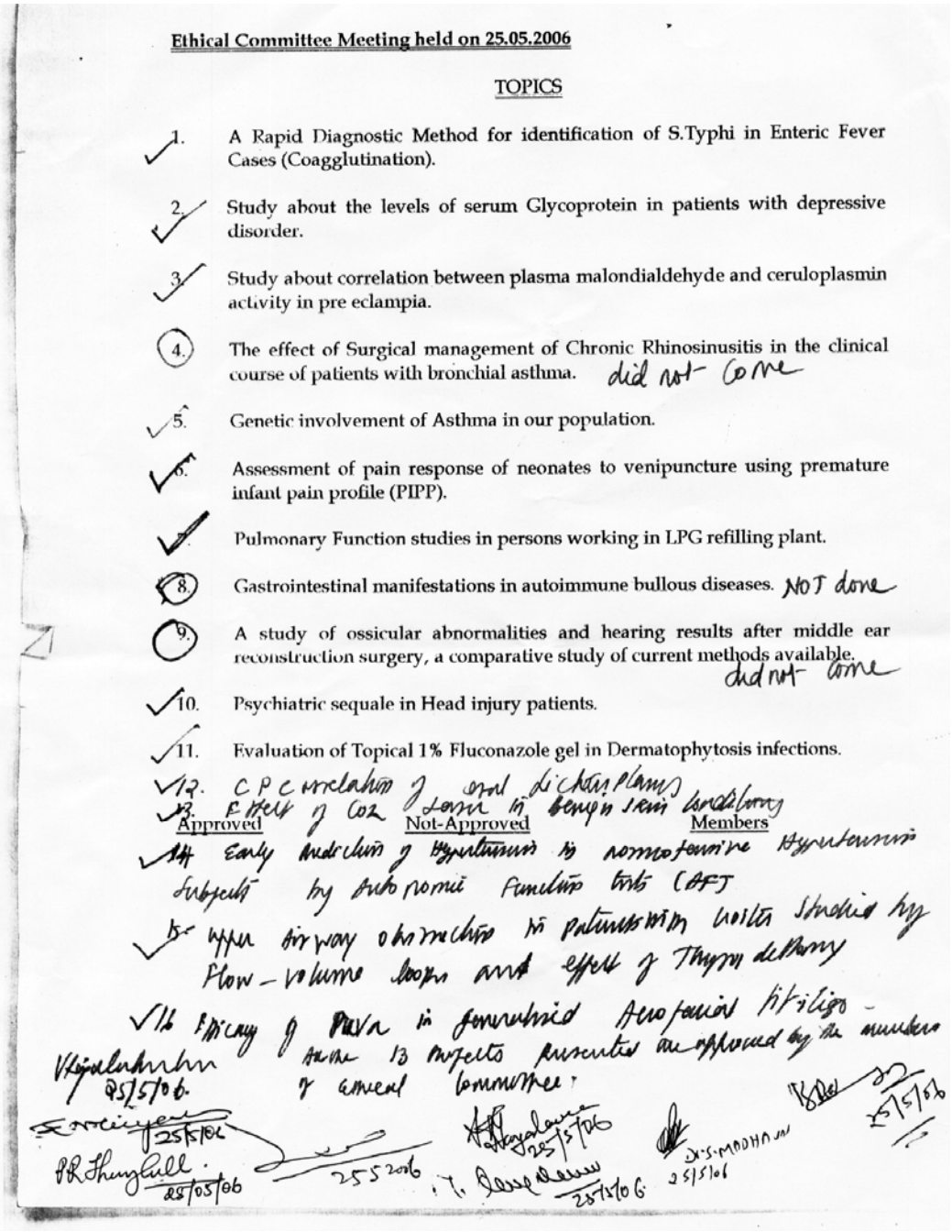
ABBREVIATIONS

SPT	-	skin prick test
PEFR	-	peak expiratory flow rate
FVC	-	forced vital capacity
FEV1	-	forced expiratory volume in one second
IgE	-	immunoglobulin E
BHR	-	bronchial hyper responsiveness.
ELISA	-	enzyme linked immunosorbent assay.
OD	-	optical density
h IgE	-	Human immunoglobulin.
AEC	-	absolute eosinophil count.
SEM	-	standard error of mean.
CD	-	cluster differentiation.
AMLase	-	acidic mammalian chitinase.
p	-	probability.
TBS	-	Tris buffered saline Tris is an abbreviation of the trishydroxymethylaminomethane
% BSA	-	Percentage bovine serum albumin
IgE-HRP conjugate	-	Immunoglobulin E conjugated with Horseradish peroxidase
OPD	-	Ortho Phenylenediamine
JMP	-	John's Macintosh Project is a statistical software.

Annexure A



Ethical Committee Meeting held on 25.05.2006

TOPICS

1. A Rapid Diagnostic Method for identification of S.Typhi in Enteric Fever Cases (Coagglutination).
2. Study about the levels of serum Glycoprotein in patients with depressive disorder.
3. Study about correlation between plasma malondialdehyde and ceruloplasmin activity in pre eclampia.
4. The effect of Surgical management of Chronic Rhinosinusitis in the clinical course of patients with bronchial asthma. did not come
5. Genetic involvement of Asthma in our population.
6. Assessment of pain response of neonates to venipuncture using premature infant pain profile (PIPP).
7. Pulmonary Function studies in persons working in LPG refilling plant.
8. Gastrointestinal manifestations in autoimmune bullous diseases. NOT done
9. A study of ossicular abnormalities and hearing results after middle ear reconstruction surgery, a comparative study of current methods available. did not come
10. Psychiatric sequale in Head injury patients.
11. Evaluation of Topical 1% Fluconazole gel in Dermatophytosis infections.
12. C P C correlation of oral lichen planus
13. Effect of Co2 Laser in benign skin conditions

Approved Not-Approved Members

14. Early detection of hypertension in normotensive hypertensive subjects by autonomic function tests (CPFT
15. Upper airway obstruction in patients with voice studied by flow-volume loops and effect of Thyroid deficiency
16. Efficacy of PUVA in generalised Acrofacial vitiligo -

Vijayalakshmi 25/5/06. Name of 13 projects presented are approved by the members of General Committee.

25/5/06 25/5/06 25/5/06 25/5/06

P.R. Thyagu 25/05/06 25/5/06 25/5/06 25/5/06

Annexure B

GENETICS OF ASTHMA

CENTER FOR BIOCHEMICAL TECHNOLOGY

CONSENT FORM FOR NORMAL/PATIENTS SAMPLES

I_____S/D/W of _____

_____

_____ Aged _____ living _____

at_____

_____ here by give my consent freely to participate in the genetic study aimed at understanding various disease processes. It has been explained to me as given in the attached information sheet the benefits and risks that the study involves; also that all the information from the questionnaire and the analysis of my blood sample will be stored anonymously and will be used as NORMAL (volunteer) / PATIENT samples for molecular genetics research. My participation in this study is entirely voluntary and I am free to withdraw from this study as and when I feel so inclined.

Signature/ Thumb impression of Normal (volunteer) / Patient.

Date:

Certified that the above consent form has been signed in my presence. The purpose for which the sample will be used has been explained to the above named Normal (volunteer)/ Patient as per the details in the information sheet. The individual is free to withdraw from the study as and when he/ she feels so inclined. He / she has given a voluntary consent to store the sample anonymously and the results of the genetic analysis on his / her blood sample confidential.

Signature of the Investigator.

Name and Designation:

Date:

_____

SAMPLE #:

DATE:

Annexure – C

INSTITUTE OF GENOMICS AND INTEGRATIVE BIOLOGY, CSIR, DELHI
GENETIC STUDIES OF ASTHMA

1. NAME
2. PERMANENT ADDRESS
3. TELEPHONE NUMBER
4. TOTAL NUMBER OF FAMILY MEMBERS
5. DOES ANY ONE IN YOUR FAMILY SUFFERS FROM ASTHMA OR ALLERGIES (PLEASE MARK Y FOR YES AND N FOR NO) Y ☐ N ☐

	AGE/SEX	ASTHMA	ALLERGY	AGE OF ONSET	RESIDENT OF CHENNAI	DEAD OR
YOU	____	☐	☐	____	☐	☐
YOUR PARENTS	____	☐	☐	____	☐	☐
YOUR BROTHER/SISTER	____	☐	☐	____	☐	☐
YOUR CHILDREN	____	☐	☐	____	☐	☐
GRANDPARENTS	____	☐	☐	____	☐	☐
MATERNAL UNCLES/AUNTS	____	☐	☐	____	☐	☐
PATERNAL UNCLES/AUNTS	____	☐	☐	____	☐	☐
MATERNAL FIRST COUSINS	____	☐	☐	____	☐	☐
PATERNAL FIRST COUSINS	____	☐	☐	____	☐	☐
OTHER RELATIVES (PLEASE SPECIFY THE RELATIONSHIP)	____	☐	☐	____	☐	☐

MASTER CHART

					SKIN PRICK TEST																
SL NO	**MODE OF LIVING**	**ECONOMY**	**AGE**	**SEX**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**IgE**
1	1	0	68	M	_	_	_	_	_	_	_	_	_	+++	_	_	++	+	_	_	_	1136.06
2	0	0	21	M	_	_	_	_	_	_	+	_	_	++	_	_	_	_	_	_	+	1437.5
3	0	0	50	M	_	_	_	_	_	_	_	_	_	_	_	_	+	++	_	_	_	485.648
4	0	0	32	F	++++	_	_	_	_	_	_	_	_	_	_	_	++	++	++++	++	_	483.648
5	1	0	46	F	_	_	_	_	_	_	_	_	_	_	_	_	+	+	+	+	_	664.65
6	1	1	19	M	+	_	_	_	_	_	_	_	+	+	_	_	_	_	_	_	_	1495
7	1	1	26	M	_	_	+	_	+	+	_	_	_	++	+++	++++	++++	++++	++	+++	_	79.41
8	0	0	12	M	+++	+	_	_	_	_	_	_	_	_	+	_	++	+++	++	_	_	409.73
9	1	0	32	M	_	_	_	_	_	_	_	+	_	+	_	_	_	++	_	_	_	141.16
10	1	1	50	F	+	_	_	_	_	_	_	_	_	++	+	+	++	+++	+	+	_	1534.61
11	0	0	31	F	++	_	_	_	_	_	_	_	_	++	++	_	++++	+++	_	++	++	48.04
12	0	0	29	F	+	_	_	_	_	_	_	_	_	_	+	_		++	_	_	_	25.83
13	1	1	28	F	+++	_	_	_	+	_	_	_	_	++	_	_	+++	++	_	_	_	37.37
14	0	1	38	F	_	_	_	+	+	_	_	_	_	_	_	_	_	_	+	_	_	37.37
15	0	0	15	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	124.34
16	0	0	29	F	+++	_	_	_	+	_	_	_	_	_	_	_	_	_	_	++	++	31.29
17	1	0	57	F	+	++	+	+	+	+	_	_	_	_	_	++	_	+	+	+	_	397.38
18	1	1	28	M	_	_	+	_	_	_	_	_	_	+++	_	_	+++	+++	++	+	_	139.19
19	1	1	18	M	_	_	_	_	_	_	_	_	_	+++	_	_	++++	+++	_	_	_	189.87
20	1	0	16	M	_	_	_	_	_	_	_	_	_	_	_	_	_	++	_	_	_	23.63
21	0	0	7	M	_	_	_	_	_	_	_	_	_	++	+	_	+	+	+	_	_	233.45
22	0	0	20	F	_	_	_	_	_	_	_	_	_	++	_	_	+++	_	+++	_	_	421.57
23	0	0	32	F	+	_	_	_	_	_	_	++	_	++	_	_	++	++	_	_	_	51.72
24	1	0	21	F	_	_	_	_	_	_	_	_	_	++	_	_	_	++	_	_	_	174.29
25	1	1	41	F	++	_	_	_	_	_	_	_	+	_	_	_	_		_	_	_	29.97
26	0	0	36	M	_	_	_	_	_	_	_	_	_	+++	_	+	_	+	_	_	_	32.76
27	1	0	33	F	_	_	_	_	_	_	_	_	_	++	_	_	++	++	_	_	_	39.7

28	1	1	30	F	_	_	_	_	_	_	_	_	_	_	_	_	_	++	_	_	+	79.58
29	1	1	37	F	_	_	_	_	+	+	_	_	_	++	++	++	++	+++	+++		_	63.74
30	1	0	40	M	+	_	_	_	_	_	_	_	_	+	_	_	_		++	+	_	51.82
31	0	0	39	M	++	_	_	_	_	_	_	_	_	+++	_	_	+++	++	_	_	_	49.92
32	0	1	37	F	_	_	_	_	_	_	_	_	_	++	_	_	+++	++	_	_	_	46.72
33	1	0	28	F	_	_	_	_	_	_	_	_	_	_	_	_	_	+++	++	_	_	35.27
34	1	0	17	F	_	_	_	_	_	_	_	_	_	++	_	_	+	_	_	_	_	125.88
35	1	0	40	F	++	+	+	++	_	_	_	_	_	++	_	_	_	+	+	+	_	61.7
36	1	0	27	M	++	_	_	_	_	+	+	+	_	++	_	_	++	_	_	_	_	163.82
37	1	1	10	F	_	_	_	_	_	_	_	+	+	_	_	_	_	_	_	_	_	60.07
38	1	0	17	F	_	_	_	_	_	_	_	_	_	++	_	_	++	++	_	_	_	49.15
39	1	1	16	F	_	_	_	_	_	_	_	_	_	_	_	_	_	+	_	_	_	106.77
40	0	0	13	M	_	_	_	_	_	_	_	_	_	+++	_	_	++	++	_	_	_	34.71
41	0	0	45	F	_	_	_	_	_	_	+	+	++	+++	_	_	+	+++	_	_	_	39.15
42	1	1	33	F	_	_	_	_	_	_	_	_	+	++	_	_	_	+	_	_	_	56.18
43	1	1	41	F	++	_	_	_	++		_	_	_	++	_	_	_	_	_	_	_	114.16
44	1	0	32	F	_	_	_	_	_	++	_	+	_	_	_	_	_	_	_	_	_	70.53
45	1	0	21	F	+	+	_	_	+	_	_	+	_	+	_	_	_	_	_	+		115.7
46	0	0	28	F	_	_	_	_	_	_	_	_	_	+	_	_	_	_	++	+++	++	105.36
47	1	0	50	F	_	+	_	_	_	_	_	_	_	++	_	++	_	++	_	_	_	86.2
48	1	0	30	M	_	++	+	_	_	_	_	_	_	++	_	_	+	++	_	++	_	132.26
49	1	0	40	F	+++	_	_	_	_	_	_	_	_	_	_	_	_		_	_	_	163.82
50	1	1	18	M	_	_	_	_	_	_	_	_	_	++	_	_	++	++	++	_	_	222.84
51	1	0	16	F	_		+	_	_	_	_	_	_	+++	_	_	+	_	_	_	_	120.44
52	1	0	18	M	++	+	_	_	_	_	_	_	_	_	_	_	++	+	_	_	_	71.75
53	1	0	21	F	_	_	_	_	_	_	+	_	_	++	_	_	++	_	_	_	_	
54	1	0	15	F	++	++	_	+	+	_	_	_	++	+++	_	_	+++	++	_	_	_	135.04
55	1	1	7	F	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	+++	++	85.24
56	0	1	35	M	_	_	_	_	_	_	_	_	_	+	_	_	++	+	+	+	+	132.76
57	0	0	36	F	++	_	_	_	_	_	_	_	_	_	_	+	+	_	+	_	_	388.39
58	0	0	40	F	+	_	_	_	_	_	_	_	_	_	_	+	++	_	_	_	_	152.14
59	0	0	14	M	_	_	_	+++	_	_	_	_	_	_	_	_	++	_	_	_	++	865.12

60	0	0	35	F	++	+	++	_	_	_	_	_	_	_	_	_	+++	_	_	_	_	126.14
61	1	1	30	M	_	_	_	_	_	_	_	_	_	+++	_	_		_	_	_	_	493.03
62	1	0	25	M	_	_	_	_	_	_	_	_	+	+++	_	_	+++	++	+	+	_	932.41
63	0	0	23	F	_	_	_	_	_	_	_	_	_	+	_	_	+	+	+	_	_	1119.25
64	1	0	14	F	_	_	_	_	_	_	_	_	_	_	_	_	_	+++	_	_	_	37.38
65	0	0	35	M	+++	++	++	++	++	++	+	_	_	_	_	_	_	++	_	_	_	1107.35
66	1	0	23	F	_	_	_	_	_	_	_	_	_	_	_	_	++	++	+	_	_	730.88
67	0	0	40	F	_	_	_	_	_	_	_	_	_	++	++		++	++	++	_	_	234.58
68	0	0	19	M	+++	_	_	_	_	_	_	_	_	_	_	++	_	++	+++		++	414.06
69	0	0	14	M	_	_	_	_	_	+	+	+	+	++	_	_	+	+	_	_	_	800.76
70	0	0	6	M	_	_	_	_	_	_	_	_	+	+	_	_	++	+	_	_	_	41.79
71	1	0	30	F	+	_	_	+	+	+	+	+	++	_	_	_	+++	++	+	+	+_	453.65
72	1	0	13	M	_	_	_	_	_	_	_	_	_	_	_	_	_		+	+	_	385.67
73	1	0	29	F	_	_	_	_	_	_	_	_	_	++	_	_	+	+	_	_	_	453.65
74	1	0	33	M	+++	_	_	_	_	_	_	_	_	_	_	_	+	_	_	_	_	389.6
75	1	0	19	F	_	_	_	_	_	_	_	_	_	+	_	_	+	_	_	_	_	627.69
76	0	0	35	F	++	_	_	_	_	_	_	_	_	+++	_	_	++	_	_	_	_	162.18
78	0	0	16	F	_	_	+	_	_	_	_	_	_	+	_	_	++	+	_	_	_	63.75
79	1	1	22	M	_	_	_	_	_	_	_	_	_	++	_	_	++	+++	+	+	_	483.37
80	1	1	18	F	+	_	_	_	_	_	_	_	+	+	_	_	_	+	+	_	_	257.97
81	1	1	18	M	++++	_	_	_	+	_	_	_	_	_	+	+	_	_	+	_	_	329.82
82	1	1	6	M	_	_	_	_	++	+	+	_	_	_	_	_	+	_	_	_	_	105.35
83	1	0	11	F	_	_	_	_	_	_	_	_	_	++	_	_	_	++	_	_	_	156.09
84	1	1	31	F	+++	++	_	_	_	_	_	_	_	_	_	+	+	_	_	_	_	65.15
85	1	1	24	F	+++	++	++	+	+	+	_	+	_	_	++	+	++	++	_	_	_	340.8
86	1	0	38	F	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	+	967.05
87	1	0	31	F	+	_	_	_	_	_	_	_	_	++	_	_	++	++	+++	_	_	252.39
88	0	1	36	F	_	_	_	_	_	_	_	+	_	+	_	_	_	+	+	_	_	199.57
89	1	0	22	F	_	_	_	_	+	_	_	+	+	+	_	_	+	+	_	+	_	161.29
90	1	0	33	M	_	_	_	_	_	+	+	+	_	+	_	_	++	++	_	+	+	

control group

					SKIN PRICK TEST																	
SL NO	**MODE OF LIVING**	**ECONOMY**	**AGE**	**SEX**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**IgE**	**SL NO**
1	_	_	44	F	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	76.36	
2	_	_	43	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	78.89	
3	_	_	57	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	62.35	
4	_	_	45	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	224.5	
5	_	_	45	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	159.8	
6	_	_	71	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	21.16	
7	_	_	38	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	71.75	
8	_	_	44	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	33.2	
9	_	_	48	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	116.1	
10	_	_	31	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	77.08	
11	_	_	36	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	211.3	
12	_	_	54	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	27.84	
13	_	_	30	F	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	29.53	
14	_	_	46	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	248.6	
15	_	_	33	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	45.36	
16	_	_	40	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	31.61	
17	_	_	58	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	115.7	

18	_	_	35	F	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	32.03	
19	_	_	13	F	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	59.27	
20	_	_	55	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	179.9	
21	_	_	46	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	151.2	
22	_	_	27	F	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	70.67	
23	_	_	40	F	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	57.24	
24	_	_	36	F	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	49.66	
25	_	_	45	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	142.7	
26	_	_	50	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	73.77	
27	_	_	32	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	137	
28	_	_	32	F	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	58.09	
29	_	_	14	F	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	65.87	
30	_	_	39	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	48.78	
31	_	_	45	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	232.5	
32	_	_	49	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	148.6	
33	_	_	26	F	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	111.3	
34	_	_	36	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	94.45	
35	_	_	20	M	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	40.74	

			EXPLANATION																					
			ECONOMY		0	POOR																		
					1	MEDIUM																		
			MODE OF LIVING		0	RURAL																		
					1	URBAN																		

+	No wheal, 3 mm flare
++	2-3 mm wheal with flare
+++	3-5 mm wheal with flare
++++	>5 mm wheal, may have pseudopodia

INDOOR ALLERGENS

House dust mite

Cockroach

Moth

Mosquito

OUTDOOR ALLERGENS (POLLENS)

Amaranthus spinosus

Brassica campestris

Prosopis juliflora

Adhatoda vasica

Azadirachta indica

Eucalyptus tereticornis

Parthenium hysterophorus

Ricinus communis

Albizzia lebbeck

Aspergilus fumigatus