IJPSR (2014), Vol. 5, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 07 November, 2013; received in revised form, 31 December, 2013; accepted, 26 March, 2014; published 01 April, 2014

STUDIES ON ALLERGENS CAUSING BRONCHIAL ASTHMA AND ALLERGIC RHINITIS

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Keywords:

Bronchial Asthma, Allergic Rhinitis, Eosinophil, Allergens, IgE (Immunoglobulin E)

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ABSTRACT: The studies on allergen causing bronchial asthma and allergic rhinitis were done by testing Immunoglobulin E level and absolute Eosinophil count to clinical patients in JEBI laboratory. The patients were from urban and rural areas, comprising both males and females in all age groups. Out of 613 patients, 523 patients were having markedly increased IgE level and 147 patients were found increased Eosinophil count. They were allergic to various allergens, most commonly to dust allergen - 383 patients, to ice cold food - 285 patients, mosquito repellent allergen - 212 patients, climatic condition in 189 patients. Hence, it is well established that modernization, change of food habits, Industrialization and luxurious life style increases the incidents of atopic diseases. There are different tests done to determine allergy and various treatments; but the best way is to slowly eliminate various factors from your life and watch for improvement in symptoms.

INTRODUCTION: Asthma and allergic disease increased worldwide in recent decades. In the world Asthma is a major public health concern. According to health organization globally it is estimated that about 235 million people are currently having asthma [1].

"Asthma is a complex disorder involving a combination of genetic and environmental interactions that leads to airway inflammation characterized by T – helper – 2 cell polarization and airway wall remodeling accompanied by extensive epithelial dysfunction" [2].

Allergen exposure is the single most powerful environmental risk factor for asthma.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(4).1416-23

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(4).1416-23

Recently there has been an increase in morbidity associated with asthma and there is strong evidence that asthma and allergic disorders are rapidly increasing in prevalence with its severity.

Hence, "asthma" a complex genetic disorder, is influenced by the interaction between genetic and environmental factors.

Genetic susceptibility Allergens, Clinical Asthma for asthma, atopic, BHR air pollutants (Reversible and Genetic heterogeneity, +Viral infections = irreversible Penetrance, phenocopy changes in airway structure seasons, age function).

Unless there is genetic susceptibility for asthma, atopy, allergens like air pollutants, smoke, viral infections, seasonal changes etc cannot cause asthma or atopic disorders.

It has been inter-nationally accepted that prevalence and severity of allergic diseases including Asthma, have increased over the last 30 years [3]. Average incidence rate is 10 to 15%.

The health cause of Asthma increases with age in the UK and has approached over £ 1 billion per Annum.

The association between Air Pollution exposure due to increased industrialization and occurrence of allergic disorder like Bronchial Asthma has been recognized.

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 the age group 5 to 11 year old children. Worldwide deaths from asthma have reached over 1, 80,000 annually.

Allergic rhinitis (AR) and asthma are both inflammatory diseases and are associated. Both asthma and allergic rhinitis are characterized by air way inflammation and blood eosinophilia.[4] Asthma is present in 20-50% of patients with AR, and up to 80% of patients with asthma have AR [5, 6].

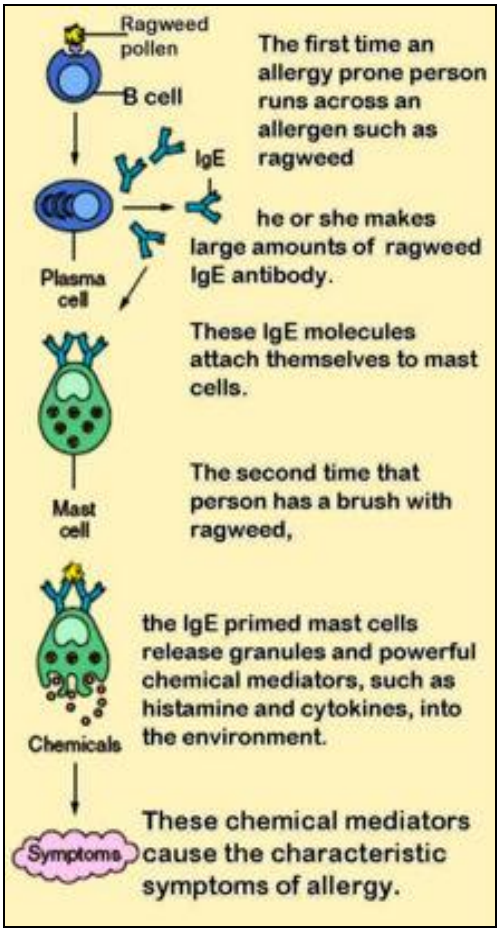
Allergy involves the production of a special class of antibody called immunoglobulin E. (IgE) which has been found only in mammals. These antibodies are bound to specific cells called mast cells, found in the skin, lungs and in many other tissues. Mast cells contain many powerful chemicals including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

In immunology, IgE is a class of antibody that plays a very 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 [7].

IgE determination is valuable in the diagnostic assessment of patients with established or suspected allergic diseases [8, 9]. Studies have been shown that the conditions such as asthma, allergic rhinitis, eczema, urticaria, atopic dermatitis and some parasitic infections leads to increased IgE levels.

In normal individuals the serum histamine binding capacity is 20 to 30 % whereas it is found that only 0 to 5% in allergic patients. This project work is done in collaboration with Dr. K. Sivanandhan who is doing this research work for the last 20years.we selected patients who have come to our clinic for treatment for atopic diseases .They have come from urban and rural areas comprising of both males and females of all age groups. Our study is based on IgE estimation and Absolute Eosinophil count to various allergens causing diseases likes allergic asthma and allergic rhinitis.

The Immunology of Allergies:



MATERIALS AND METHOD:

Collection of sample: Blood samples are collected by vein puncture allowed to clot and separated the serum by centrifugation at room temperature. If sera cannot be assayed immediately, then they will be stored at 2-8^{0}C for a week of period, avoiding repeated freezing and thawing of serum samples.

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. 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 brought to room temperature; used new sterile disposable tips for each specimen. Secure the desired number of coated wells in the holder. Dispensed 10uL of standards, controls or serum samples into appropriate wells, immediately added 100uL of UBI zero standard diluents into each well, then incubated for 30 minutes at room temperature. Then removed from the incubation mixture and rinsed the wells 5 times with tap water (300uL) thoroughly; dispensed 100uLof enzyme conjugate to each well and again it is incubated for 30 minutes; removed the incubation mixture and rinsed with water five times. Dispensed 100uL of solution A and then 100uL of solution B into each well and incubated for 10 minutes at room temperature. Adding 50uL of stop solution and stopped the reaction and then read the O.D at 450 nm with a micro well reader

Washed the micro wells and removed water thoroughly. Then pipetted all reagents and samples into the 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. We kept reagents, samples and needed wells ready and assigned. It ensured the equal elapsed time for each Pipetting without interruption. For the same reason, the size of the assay run each time is also limited. It is suggested to run not more than 20 patients with a set of reference standards in duplicate.

Calculation: Micro well reader capable of determining at 450 nm is used. The IgE value of patient is obtained as follows:

Plotted the concentration (X) of reference standard against absorbance (Y) on full logarithmic paper. Obtained the value of patient IgE by reference to the standard curve.

Expected value: Serum IgE may vary as a result of season of that geographical location, diet and also the year. It is recommended that laboratories should establish their expected normal range from time to time. Studies of the expected concentration of IgE in a population of healthy, non-allergic individuals are complicated by the fact that some individuals 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: up to 2000 IU/mL; Specimen required: 2mL random serum sample

Absolute Eosinophil Count: It is important to do a direct total or absolute eosinophil count to get an accurate value. The diluting fluid used to stain the eosinophils distinctly and lyses the other types of Leucocytes and red cells. We used most commonly used diluting fluid Hingleman's Solutions which is based on this principle. This diluting fluid contains yellow eosin (0.5 gms) 95% phenol (0.5 ml) Formalin (0.5 ml) distilled water (99.0 ml)., to stain eosinophils to view clearly in the Fuchs-Rosenthal counting chamber to count under low power i.e. 10X objective and 10X eye piece. For reasonably accurate count we counted at least 100 cells. Normal count is 40 – 440 / Cumm. The results were given in Table 5 and chart 5.

RESULTS AND DISCUSSIONS: Studies on allergens causing bronchial asthma and allergic rhinitis to the patients who attend our allergic clinic regularly shows that different allergens which cause them to allergic Asthma and Allergic Rhinitis.

Dust allergies are usually allergies to dust mites, dust particles, pollen, mold, dander and even chemicals in the atmosphere. Among all this, Dust allergies are quite quick to show effects.

Dust can be formed as a result of anything and is rarely a reflection on cleaning abilities. Dust can be created by pets, furniture, location of living, plants in the house and many other seasonal factors.[10] a fleck of dust can contain everything from human skin to pet dander, other living organisms like bacteria and fungi and of course dust mites. Dust mites are one of the primary causes of dust allergy[11].

In warm and humid climates dust mites are common and are found all over the house. Invisible to the naked eye, these mites live off dead skin of humans and animals. Dust mites are common cause for allergic rhinitis, apart from triggering the attacks of asthma [12].

Cold or a runny nose throughout the year indicates the people are actually allergic to something [13].

Allergic rhinitis in childhood is considered incurable and may impair quality of life and school performance. Therapy includes mainly allergen avoidance and immunosuppressant drugs [14]. But, now it is being proved that allergic disorders like allergic rhinitis, bronchial asthma etc, can be cured by increasing the level of serum Histamine Binding Capacity [15].

Exposure to Tobacco Smoke is most commonly responsible for increased cases of asthma episodes in adolescents. There is also evidence which suggests that adolescents exposed to smoke also show decreased pulmonary function tests [16].

The mosquito repellents are toxic chemicals. It may cause allergic and respiratory problems even through in a low safe concentration [17].

The Temperature of food and beverages can have on effect on asthmatic patients. Cold carbonated beverages increase the asthma attack. Over eating and gas causing food items trigger asthma attack i.e. Foods like Beans, Cucumbers, Cabbage, Brussels sprouts, cauliflower, broccoli.

Warmer temperature, rising sea levels closely associated with global climatic changes are also responsible for increase in allergies.

Perfumes are frequent triggers for an asthma attack. Scent and strong smells irritating the respiratory system, can cause difficulty of breathing, coughing and wheezing. Fragrances are added in cleaning agents, personal care and cosmetic items, petroleum products, room deodorants and even in prepared foods. Approximately 30 percent of the population report sensitivities to scented products, and as of 2010, more than 5,000 fragrances are included in fragrances and cosmetics alone, according to The American Academy of Dermatology [18].

We have made studies over 613 patients who have either allergic asthma or allergic rhinitis and who have come to our clinic for treatment. Our study is based on IgE estimation and AEC count.

TABLE 1: ALLERGENS CAUSING BRONCHIAL ASTHMA AND ALLERGIC RHINITIS

Allergens	No. of Persons
Dust	383
Smoke	182
Pollen Grains	6
Mosquito Repellent	212
Scented and Strong Smells	89
Ice Cold Food	285
Milk & Its Products	89
Fruit Juice	10
Fruits	110
Sea Food	5
Mutton, Chicken and Egg	10
Exercise	27
Crying	1
Climate	189
Entomological Organism	10
Fan Air	5
Mushroom	1
Head Bath	59
Hereditary	5
Viral Infection	1
Alcohol	3
Pesticides	2

We found out the different causative agents which cause either allergic asthma or allergic rhinitis i.e. Dust, Smoke, Pollen Grains, Mosquito repellent, Scented and Strong smells, ice Cold Foods, etc., (see Ref. **Table 1, Chart 1**). In our investigation, the number of affected females are 271 and males are 324 (see Ref. **Table 2, Chart 2**). Area wise estimation result is 296 and 317 respectively in the rural and urban area but urban area is slightly higher than rural area (see Ref. **Table 3, Chart 3**). According to elevated IgE level, numbers of

affected persons are 523 out of 613 (see Ref. **Table 4, Chart 4**). And increased Eosinophil count is seen in 147 patients out of 613, (i.e. 24% - see Ref. **Table 5, Chart 5**). The health cause of Asthma is increased with age groups. Adults are more affected than the children. In our studies, more males are affected than females (see Ref. **Table 6, Chart 6**). And duration of disease for more than one year is higher (see Ref. **Table 7, Chart 7**).

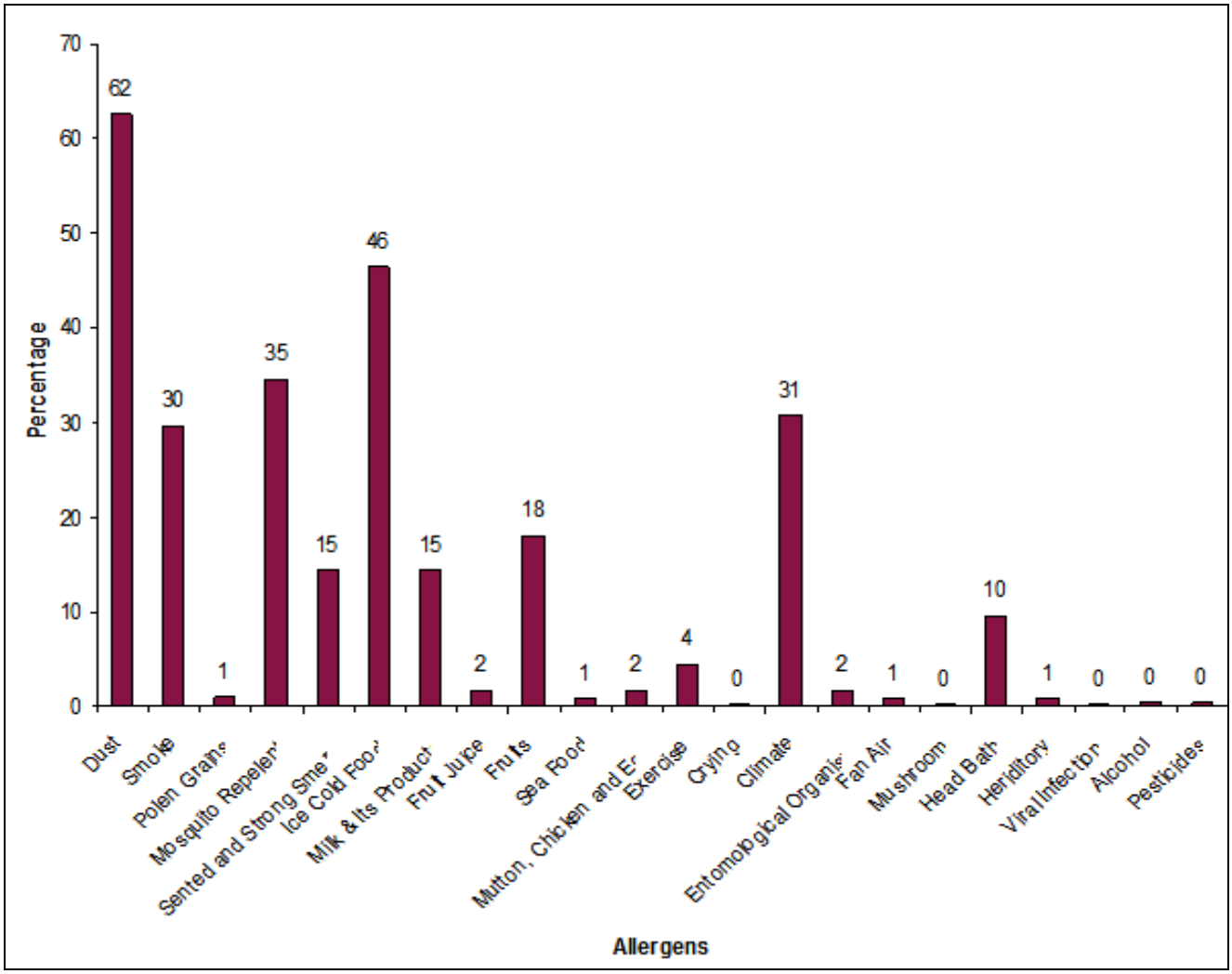


CHART 1:

TABLE 2: GENDER- WISE CLASSIFICATION

Gender	Nos.
Male	342
Female	271

TABLE 3: AREA- WISE CLASSIFICATION

Area	Nos.
Rural	296
Urban	317

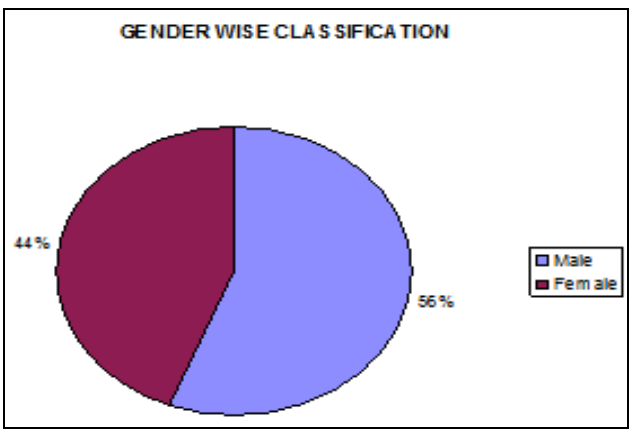


CHART 2:

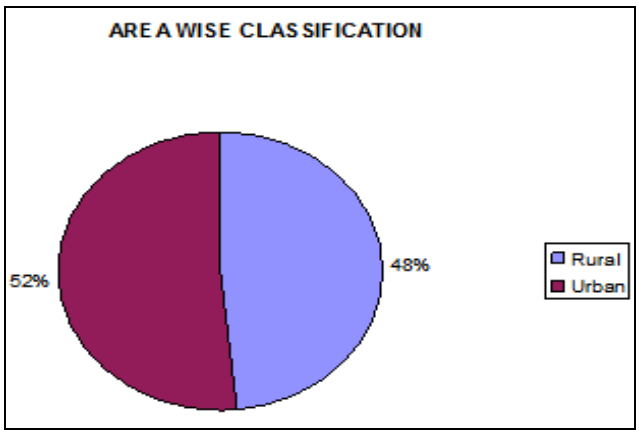


CHART 3

TABLE 4: IgE BASED CLASSIFICATION

IgE	Nos.
<100	90
100 - 200	99
201 - 500	199
501 - 1000	172

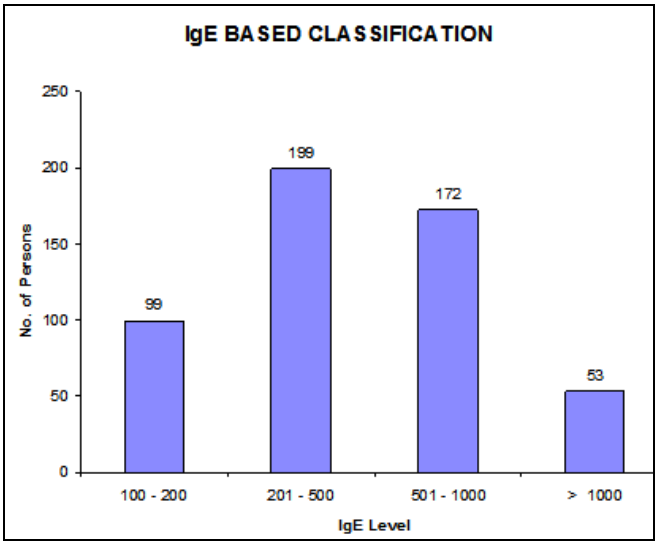


CHART 4:

TABLE 5: ABSOLUTE EOSINOPHIL COUNT

Eosinophil	Nos.
> 440 cells/cumm	147

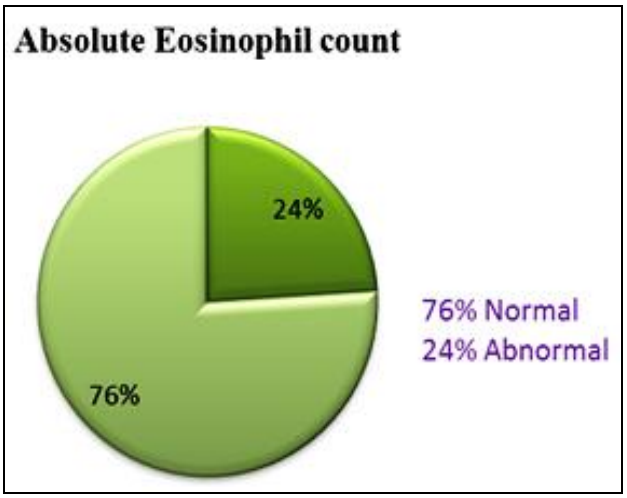


CHART 5:

TABLE 6: AGE -WISE CLASSIFICATION

Age	No. of Male	No. of Female	Total
0 - 5 Yrs.	31	18	49
6 - 10 Yrs.	42	30	72
11 - 15 Yrs.	34	25	59
16 - 25 Yrs.	45	47	92
Above 25 Yrs.	190	151	341

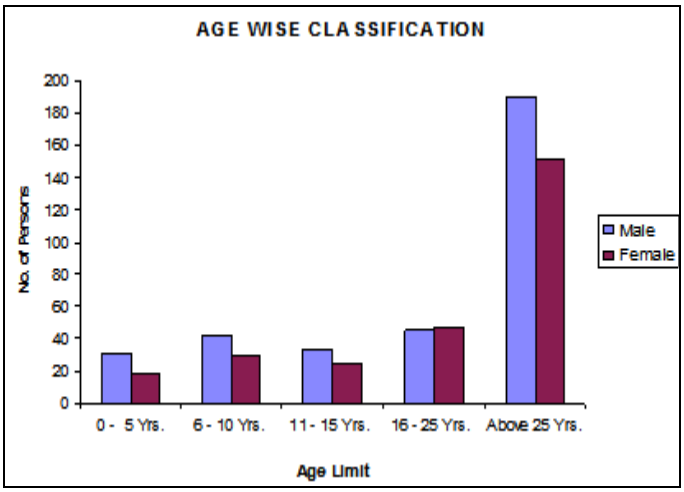


CHART 6:

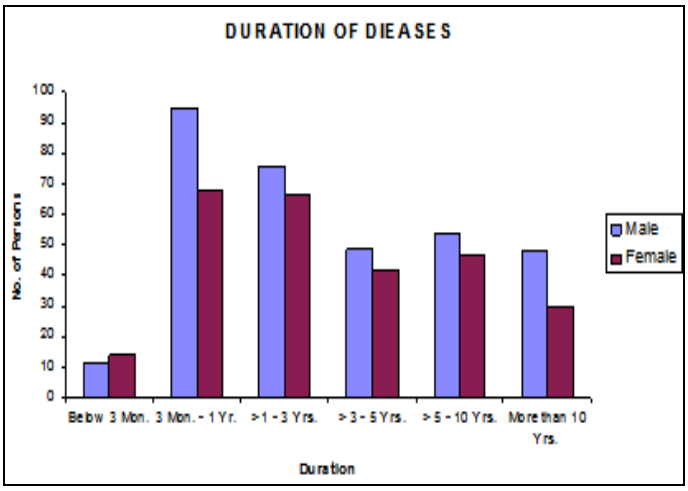


CHART 7:

TABLE 7: DURATION OF DISEASE

Duration	No. of Male	No. of Female	Total
Below 3 Mon.	12	14	26
3 Mon. - 1 Yr.	102	73	175
> 1 - 3 Yrs.	76	66	142
> 3 - 5 Yrs.	49	42	91
> 5 - 10 Yrs.	54	47	101
More than 10 Yrs.	48	30	78

In our study we found out the following facts:

i) Cutting trees, converting agricultural land to housing plot, industrialization, increasing motor vehicles, unventilated Air conditioned rooms, Air conditioned travels, Various cosmetics, hair dye, and lip stick

,food habit i.e. fast food, over eating, are all responsible for allergy.

ii) Dust plays a major role in causing allergic asthma and allergic rhinitis.

iii) Ice cold food (Cool drinks, Ice Cream, Chill water, refrigerated food items) are another major reason for causing allergic asthma and allergic rhinitis.

iv) Mosquito repellants widely used in all houses, is also another major factor for causing atopic diseases.

v) Global climatic condition has an impact on asthma patients.

vi) Various Smokes (Agarpathi, Samparani, and vehicle smoke, and cigarette, industrial, crackers) are responsible for the atopic diseases.

vii) Scented and Strong smells i.e., Jasmine, Powder, Paints, Cosmetics, Cow Dung Smell and various scented products induce asthmatic attacks.

Apart from the various factors mentioned above, pollen grains, milk and its products,

fruit juices, fruits, sea food, mutton, chicken and egg, exercise, crying, entomological organism, fan air, mushroom, head bath, hereditary, viral infection, alcohol, pesticides are also responsible for causing allergic asthma and allergic rhinitis to some extent.

CONCLUSION: In our studies, we have found out that the dust play a major role for causing atopic diseases. The Ice cold food, Mosquito repellent and various smoke are also responsible for causing atopic diseases. It indicates that modernization, industrialization and luxurious life styles are main cause for Asthma and Allergic Rhinitis.

Therefore to what extent we avoid the causative factors, we can prevent the occurrence of the atopic diseases. Because, prevention is far better than cure, isn't it?

ACKNOWLEDGEMENT: It is my honour and great privilege to thank **Dr. R. Selvakumaran,** Mutthayammal Arts and Science College, Namakkal for his continuous support, systematic guidance and invaluable suggestions rendered throughout my project work. I am deeply grateful for the time, interest and energy he has devoted toward helping me to achieve success in my project.

It is my great pleasure to thank Dr. K. Sivanandhan, M.B.B.S., DCH. M.S. (Research), Sri Ragavendra Clinic, Pollachi for suggesting me to study on Allergic Asthma and Allergic Rhinitis. He has been work in Allergic Asthma for last 20 years. His paper about Allergic Asthma – A curable disease has been accepted by World Allergic Organization.

It is my honour and privilege to thank Dr. A. Michael, M.Sc., DMT., Ph.D., Associate Professor and Head, Department of Microbiology, PSG College of Arts and Science, Coimbatore for his support and invaluable suggestions rendered throughout the study.

My sincere thanks also to Dr. M. Palanisamy**,** Head, Department of Micro Biology, Karpagam University, Coimbatore for his guidance and support rendered throughout my project work.

REFERENCES:

1. Sewell. D.A., Hammersley. V.S., Devereux. G., Robertson. A., Stoddart. A., Weir. C., Worth. A and Sheikh. A., Investigating the effectiveness of the Mediterranean diet in pregnant women for the primary prevention of asthma and allergy in high-risk infants: protocol for a pilot randomized controlled trial., Biomed central., 2013, 14:173
2. Sayers, B. Beghe, J. Holloway B.S.T. Holgat genetics of Asthma: What's new?
3. Anonymous, world wide variation in prevalence of symptoms of asthma, allergic rhino conjunctivitis, and atopic eczema : ISAAC Lancet 1998; 351 (9111) : 1225 – 32
4. Cindy MA de Bot, Heleen Moed, Patrick JE Bindels, Roy Gerth van Wijk, Marjolein Y Berger, Hans de Groot, Johannes C de Jongste, Johannes C van der Wouden, Exhaled nitric oxide measures allergy not symptoms in children with allergic rhinitis in primary care: a prospective cross-sectional and longitudinal cohort study, Prim Care Respiratory Journals 2013; 22(1) : 44-50.
5. J Delgado, I Dávila, J Domínguez-Ortega, S Quirce, E Martí-Guadaño, A Valero, Quality of Life in Patients With Respiratory Allergy is influenced by the Causative Allergen, J Investig Allergol Clin Immunol 2013; Vol. 23(5): 309-314.
6. Cunill.A.S., Bartra.J., Dalmau.G., Tella.R., Botey.E., Raga.E., Valero.A., Prevalence of Asthma and Severity of Allergic Rhinitis Comparing 2 Perennial Allergens: House Dust Mites and *Parietaria judaica* Pollen, J Investig Allergol Clin Immunol 2013; Vol. 23(3):145-151.
7. Immunology by Kuby: Pg. 96: 6th Edition.

8. Holt P. G. Macaubas C, Stumbles PA, Sly PD. Nature 1999; 402: B-12-17.
9. Holgate ST. The epidemic of allergy and asthma. Nature 1999; 402: 132 - 4.
10. Kram. A.A., Dmowsk. E., Spectrum and Concentration of Culturable Fungi in House Dust from Flats in Warsaw, Poland. Aerosol and Air Quality Research, 2013, 13: 1438–1447.
11. Lee. J.H., Kim. S.H., Lee. W.Y., Yong. S.J., Shin. K.C., Lee. M.K., Kim. C.W. Immunotherapy -2079. Comparison of house dust mite specific IgE and IgG4 between patients receiving rush immunotheraopy and conventional immunotherapy, World Allergy Organization Journal 2013, 6(Suppl 1):P161.
12. Fereidouni.M., Fereidouni.F., Hadian.M., Asghari.Z., Zojaji.S.M., The Level of Mite Dermatophagoides' Allergens (Der-p 1 and Der-f 1) in Birjand, Zahedan, Journal of Research in Medical Sciences; 2011; Jul; 16(4): 38-40.
13. www.medicalhealthtests.com
14. Yonekura.S., Okamoto.Y., Sakurai.D., Horiguchi.S., Hanazawa.T., Nakano.A., Kudou.F., Nakamaru.Y., Honda.K., Hoshioka.A., Shimojo.N. and Kohno.Y. Sublingual Immunotherapy with House, Dust Extract for House Dust-Mite Allergic Rhinitis in Children, Allergology International. 2010; 59:381-388.
15. Epidemiological study of Asthma in and around Pollachi. Sivanandhan.K. Karpagam University, Coimbatore, December 2011.
16. http://www.aafa.org
17. http://dmohankumar.wordpress.com
18. http://www.livestrong.com

How to cite this article:
Manohar S, Sivanandhan K, Michael A and Selvakumaran R: Studies on allergens causing bronchial asthma and allergic rhinitis. *Int J Pharm Sci Res* 2014; 5(4): 1416-23.doi: 10.13040/IJPSR.0975-8232.5(4).1416-23