Prevalence of sensitization to mould and yeast allergens in Egyptian patients with respiratory allergy

Thesis
Submitted for partial fulfillment of M.D. degree in Medicine
By

Medhat Maher Abd El-latif Elamawy
M.Sc.

Faculty of Medicine, Banha University

Supervisors

Dr. Nabil Elsayed Khattab
Professor of Medicine
Faculty of Medicine
Banha University

Dr. Atef Ahmed Ibrahim
Professor of Medicine
Faculty of Medicine
Banha University

Dr. Maged Mohamed Refaat
Professor of Medicine, Allergy and Immunology
Faculty of Medicine
Ain Shams University

Dr. Mohamed Nazmy Farres
Professor of Medicine, Allergy and Immunology
Faculty of Medicine
Ain Shams University

Faculty of Medicine
Banha University
2017
الحمد لله رب العالمين
الرحمن الرحيم
ملك يوم الدين
إياك نعبدو وإياك نستعين
اهدينا الضرط الامستقيمة
صرط الدين أنعمت عليهم
غير المغضوب عليهم
ولا الضالين
Acknowledgement

First and for most my deep thanks "ALLAH" the most merciful who gave me the power to carry the present work honestly and faithfully.

I am sincerely grateful to Dr. Nabil Elsayed Khattab Professor of Internal Medicine, Faculty of Medicine, Banha University for his continuous encouragement in finishing this work. With his kind knowledge and enthusiasm, I was able to complete this work.

I would like to express my deepest sense of gratitude and obligation to Dr. Atef Ahmed Ibrahim Professor of Internal Medicine, Faculty of Medicine, Banha University for his constant guidance and valuable suggestion during preparation of this work.

More and more thanks to Dr. Maged Mohamed Refaat Professor of Internal Medicine, Allergy and Clinical Immunology, Faculty of Medicine, Ain Shams University for his great support; he is really my mentor with his careful supervision which helped me to overcome all difficulties.

Thanks to Dr. Mohamed Nazmy Farres Professor of Internal Medicine, Allergy and Clinical Immunology, Faculty of Medicine, Ain Shams University for his helpful cooperation and inexhaustible patience throughout the entire work.

Also I would like to express my great gratitude to Dr. Ali Elsayed Ali Assistant Professor of Internal Medicine, Faculty of Medicine, Banha University for his generous guidance and reading the manuscript to provide me with efficient and constructive suggestion.

Finally, my deepest gratitude to all professors and staff members of internal medicine department, Faculty of Medicine, Banha University and Allergy and Clinical immunology unit's staff members, Ain Shams faculty of medicine for their help and encouragement and every person who helped me in completing this work.
# Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of abbreviations</td>
<td>I</td>
</tr>
<tr>
<td>List of tables</td>
<td>III</td>
</tr>
<tr>
<td>List of figures</td>
<td>V</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Aim of work</td>
<td>4</td>
</tr>
<tr>
<td>Review of literature</td>
<td>5</td>
</tr>
<tr>
<td>Chapter 1: Allergology origin and allergic response pathogenesis:</td>
<td>6</td>
</tr>
<tr>
<td>Brief history of allergology origin</td>
<td></td>
</tr>
<tr>
<td>Hypersensitivity reactions classifications.</td>
<td></td>
</tr>
<tr>
<td>Allergic reactions etiology and Pathophysiological mechanism</td>
<td></td>
</tr>
<tr>
<td>Chapter 2: Epidemiology:</td>
<td>13</td>
</tr>
<tr>
<td>A global problem in children and adults</td>
<td></td>
</tr>
<tr>
<td>Prevalence of allergic rhinitis worldwide.</td>
<td></td>
</tr>
<tr>
<td>Prevalence of asthma worldwide.</td>
<td></td>
</tr>
<tr>
<td>Egypt prevalence of respiratory allergic diseases.</td>
<td></td>
</tr>
<tr>
<td>Chapter 3: Respiratory allergic diseases:</td>
<td>20</td>
</tr>
<tr>
<td>One airway, one disease:</td>
<td></td>
</tr>
<tr>
<td>Is it a link between allergic rhinitis and allergic asthma?</td>
<td></td>
</tr>
<tr>
<td>The Bidirectional Link between the Nose &amp; the Bronchi: experimental evidences.</td>
<td></td>
</tr>
<tr>
<td>Effects of Treatment on Rhinitis &amp; Asthma</td>
<td></td>
</tr>
<tr>
<td>Bronchial asthma:</td>
<td></td>
</tr>
<tr>
<td>Definitions</td>
<td></td>
</tr>
<tr>
<td>Asthma phenotypes versus endotypes</td>
<td></td>
</tr>
<tr>
<td>Genetic and Immunological insights</td>
<td></td>
</tr>
<tr>
<td>-----------------------------------</td>
<td></td>
</tr>
<tr>
<td>Clinical assessment and Investigations</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
</tr>
<tr>
<td>Allergic Rhinitis:</td>
<td></td>
</tr>
<tr>
<td>Definition and classification:</td>
<td></td>
</tr>
<tr>
<td>Risk factors:</td>
<td></td>
</tr>
<tr>
<td>Pathophysiology of allergic rhinitis</td>
<td></td>
</tr>
<tr>
<td>Diagnosis of allergic rhinitis</td>
<td></td>
</tr>
<tr>
<td>Overview of management:</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 4:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeroallergen and respiratory allergic diseases:</td>
</tr>
<tr>
<td>Introduction</td>
</tr>
<tr>
<td>Indoor and outdoor aeroallergens</td>
</tr>
<tr>
<td>Fungal Allergens</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory fungal allergy:</td>
</tr>
<tr>
<td>Fungal allergy in respiratory allergic diseases</td>
</tr>
<tr>
<td>Mould sensitivity and respiratory allergy severity</td>
</tr>
<tr>
<td>Mechanism of fungal respiratory allergy</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patients and methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statistical methods</td>
</tr>
<tr>
<td>Results</td>
</tr>
<tr>
<td>Discussion</td>
</tr>
<tr>
<td>Summary and conclusions</td>
</tr>
<tr>
<td>Recommendations</td>
</tr>
<tr>
<td>References</td>
</tr>
<tr>
<td>Arabic summary</td>
</tr>
<tr>
<td>74</td>
</tr>
<tr>
<td>80</td>
</tr>
<tr>
<td>81</td>
</tr>
<tr>
<td>96</td>
</tr>
<tr>
<td>106</td>
</tr>
<tr>
<td>111</td>
</tr>
<tr>
<td>113</td>
</tr>
<tr>
<td>(1)</td>
</tr>
</tbody>
</table>
# List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAFS</td>
<td>Asthma Associated with Fungal Sensitization</td>
</tr>
<tr>
<td>ABPA</td>
<td>Allergic Bronchopulmonary Aspergillosis</td>
</tr>
<tr>
<td>ABPA-CB</td>
<td>ABPA with Central Bronchiectasis</td>
</tr>
<tr>
<td>ABPA-S</td>
<td>ABPA diagnosed Serologically</td>
</tr>
<tr>
<td>ABPM</td>
<td>Allergic Bronchopulmonary Mycosis</td>
</tr>
<tr>
<td>AFS</td>
<td>Allergic Fungal Sinusitis</td>
</tr>
<tr>
<td>AH</td>
<td>Aspergillus Hypersensitivity</td>
</tr>
<tr>
<td>AHR</td>
<td>Airway Hyperresponsiveness</td>
</tr>
<tr>
<td>APCs</td>
<td>Antigen Presenting Cells</td>
</tr>
<tr>
<td>AR</td>
<td>Allergic Rhinitis</td>
</tr>
<tr>
<td>ARIA</td>
<td>Allergic Rhinitis and Its Impact on Asthma</td>
</tr>
<tr>
<td>ASMA</td>
<td>Asthma Self-Management for Adolescents approach</td>
</tr>
<tr>
<td>ATS</td>
<td>American Thoracic Society</td>
</tr>
<tr>
<td>BHR</td>
<td>Bronchial Hyperresponsiveness</td>
</tr>
<tr>
<td>CBC</td>
<td>Complete Blood Count</td>
</tr>
<tr>
<td>EAACI</td>
<td>European Academy of Allergy and Clinical Immunology</td>
</tr>
<tr>
<td>EBC</td>
<td>Exhaled Breath Condensate</td>
</tr>
<tr>
<td>ECP</td>
<td>Eosinophil Cationic Protein</td>
</tr>
<tr>
<td>ELF</td>
<td>Epithelial Lining Fluid</td>
</tr>
<tr>
<td>e NO</td>
<td>Exhaled Nitric Oxide Values</td>
</tr>
<tr>
<td>FAST</td>
<td>Fungal Asthma Sensitization Trial</td>
</tr>
<tr>
<td>GINA</td>
<td>Global Initiative of Asthma</td>
</tr>
<tr>
<td>HARP</td>
<td>Helping Asthma in Real Patients</td>
</tr>
<tr>
<td>HDMs</td>
<td>House Dust Mites</td>
</tr>
<tr>
<td>HIF</td>
<td>Hypoxia-Inducible Factor</td>
</tr>
<tr>
<td>HRCT</td>
<td>High-Resolution computed tomography</td>
</tr>
<tr>
<td>Acronym</td>
<td>Term</td>
</tr>
<tr>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>ICS</td>
<td>Inhaled Corticosteroids</td>
</tr>
<tr>
<td>IPCRG</td>
<td>International Primary Care Respiratory Group</td>
</tr>
<tr>
<td>IRC</td>
<td>International Rhinitis Consensus</td>
</tr>
<tr>
<td>ISAAC</td>
<td>International Study of Asthma and Allergies in Childhood</td>
</tr>
<tr>
<td>IUUIS</td>
<td>International Union of Immunological Societies</td>
</tr>
<tr>
<td>LABAs</td>
<td>Long-Acting β2 Agonists</td>
</tr>
<tr>
<td>MALT</td>
<td>Mucosal-Associated Lymphoid Tissue</td>
</tr>
<tr>
<td>MAST</td>
<td>Multiple Allergen Simultaneous Tests</td>
</tr>
<tr>
<td>MHC</td>
<td>Major Histocompatibility Complex</td>
</tr>
<tr>
<td>PAMP</td>
<td>Pathogen-Associated Molecular Patterns</td>
</tr>
<tr>
<td>PAR</td>
<td>Protease-Activated Receptors</td>
</tr>
<tr>
<td>PATS</td>
<td>Preventive Allergy Treatment Study</td>
</tr>
<tr>
<td>PEF</td>
<td>Peak Expiratory Flow</td>
</tr>
<tr>
<td>PNIF</td>
<td>Peak Nasal Inspiratory Flow</td>
</tr>
<tr>
<td>PG(E2)</td>
<td>Prostaglandin E2</td>
</tr>
<tr>
<td>RAST</td>
<td>Radioallergosorbent Test</td>
</tr>
<tr>
<td>SAFS</td>
<td>Severe Asthma with Fungal Sensitization</td>
</tr>
<tr>
<td>SARP</td>
<td>Severe Asthma Research Program</td>
</tr>
<tr>
<td>SBP</td>
<td>Segmental Bronchial Provocation</td>
</tr>
<tr>
<td>S-IgA</td>
<td>Secretory Immunoglobulin A</td>
</tr>
<tr>
<td>SLIT</td>
<td>Sublingual immunotherapy</td>
</tr>
<tr>
<td>SPT</td>
<td>Skin Prick Test</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-Like Receptors</td>
</tr>
<tr>
<td>Treg</td>
<td>Regulatory T cell</td>
</tr>
<tr>
<td>VOCs</td>
<td>Volatile Organic Compounds</td>
</tr>
<tr>
<td>WAO</td>
<td>World Allergy Organization</td>
</tr>
</tbody>
</table>
List of tables:

List of tables in review of literatures section:

<table>
<thead>
<tr>
<th>Tables:</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1: Prevalence of asthma worldwide (Valovirta, 2001).</td>
<td>17</td>
</tr>
</tbody>
</table>

List of tables in results section:

<table>
<thead>
<tr>
<th>Tables:</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table (1): Demographic distribution of studied patients with respiratory allergic diseases and various age groups (n=200 patients).</td>
<td>85</td>
</tr>
<tr>
<td>Table (2): Distributions of gender in studied population (n =200 patients).</td>
<td>85</td>
</tr>
<tr>
<td>Table (3): Distributions of studied patients regarding the type of respiratory allergy and prevalence (n=200 patients).</td>
<td>85</td>
</tr>
<tr>
<td>Table (4): Categorization of allergic rhinitis patients and allergic rhinitis with allergic bronchial asthma according to severity of rhinitis (n: 120 patients).</td>
<td>87</td>
</tr>
<tr>
<td>Table (5): Patient's categorization based on the degree of asthma control (n: 106)</td>
<td>88</td>
</tr>
<tr>
<td>Table (6): Results of skin prick test in studied patients (n = 200 patients).</td>
<td>89</td>
</tr>
</tbody>
</table>
**Table (7):**
Positive SPT regarding fungal sensitivity (n = 148 patients).

**Table (8):**
Results of skin prick tests (SPT) in various age groups of studied patients (n = 200 patients).

**Table (9):**
Results of SPT to common fungal allergens in studied population (n=62 patients).

**Table (10):**
Results of SPT to other non-fungal allergens from positive group (n=148).

**Table (11):**
Results of SPT to common allergens in studied patients regarding to predominant atopic symptoms.

**Table (12):**
Fungal sensitivity and level of asthma control.
## List of figures:

### List of figures in review of literatures section:

<table>
<thead>
<tr>
<th>Figures:</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Figure 1:</strong></td>
<td>12</td>
</tr>
<tr>
<td>Illustrated Pathways that Lead to Acute and Chronic Allergic Reactions (Kay, 2001)</td>
<td></td>
</tr>
<tr>
<td><strong>Figure 2:</strong></td>
<td>14</td>
</tr>
<tr>
<td>Prevalence of allergies (not only respiratory allergies) in the countries surveyed (Valovirta, 2001).</td>
<td></td>
</tr>
<tr>
<td><strong>Figure 3:</strong></td>
<td>32</td>
</tr>
<tr>
<td>Pathogenesis of asthma: Antigen presentation by the dendritic cell with the lymphocyte and cytokine response leading to airway inflammation and asthma symptoms</td>
<td></td>
</tr>
<tr>
<td><strong>Figure 4:</strong></td>
<td>34</td>
</tr>
<tr>
<td>Hygiene hypothesis, Image adapted from: (Busse and Lemanske, 2001)</td>
<td></td>
</tr>
<tr>
<td><strong>Figure 5:</strong></td>
<td>39</td>
</tr>
<tr>
<td>(a) Healthy airways produce a relatively low level of NO under the control of chemical messengers not affected by the use of ICSs. <em>(b)</em> In the asthmatic airway, inflammatory mediators, which can be downregulated by ICSs, are released in response to asthma triggers. These mediators increase the level of inducible NO synthase (iNOS), which in turn, increases the production of NO.</td>
<td></td>
</tr>
<tr>
<td><strong>Figure 6:</strong></td>
<td>46</td>
</tr>
<tr>
<td>Skin prick test and intradermal test (Adopted from Korean Academy of Pediatric Allergy and Respiratory Disease).</td>
<td></td>
</tr>
<tr>
<td><strong>Figure 7:</strong></td>
<td>57</td>
</tr>
<tr>
<td>Alternaria alternata showing branched acropetal chains and multicelled</td>
<td></td>
</tr>
<tr>
<td><strong>Figure 8:</strong></td>
<td>58</td>
</tr>
<tr>
<td>Conidiophores of Aspergillus. It may be Aspergillus fumigatus.</td>
<td></td>
</tr>
<tr>
<td><strong>Figure 9:</strong></td>
<td>59</td>
</tr>
<tr>
<td>Cladosporium</td>
<td></td>
</tr>
</tbody>
</table>
**Figure 10:**

Penicillium citrinum; distinguishing features of Penicillium 'species' is much more evident at 400X. Typical "fingers" made up of the metulae and phialide structures from which chains of conidia extend.

**Figure 11:**

Natural history of asthma in relation to fungal sensitization, with asthma at one end and Allergic Bronchopulmonary Aspergillosis at the other end. AAFS (Asthma Associated with Fungal Sensitization); ABPA-CB (Allergic Bronchopulmonary Aspergillosis with Central Bronchiectasis); ABPA-S (Seropositive Allergic Bronchopulmonary Aspergillosis); SAFS (Severe Asthma with Fungal Sensitization); Th2 (T-helper type 2 cell) (Agarwal R., 2011).
### List of figures in results section:

<table>
<thead>
<tr>
<th>Figures:</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure (1):</td>
<td>86</td>
</tr>
<tr>
<td>Percentage of various types of respiratory allergy in the studied population (n= 200 patients).</td>
<td>86</td>
</tr>
<tr>
<td>Figure (2):</td>
<td>87</td>
</tr>
<tr>
<td>Percentage of combined allergic bronchial asthma and allergic rhinitis among patients with allergic rhinitis (n=120 patients).</td>
<td>87</td>
</tr>
<tr>
<td>Figure (3):</td>
<td>88</td>
</tr>
<tr>
<td>Percentage of combined allergic bronchial asthma and allergic rhinitis among patients with allergic bronchial asthma (n= 106 patients).</td>
<td>88</td>
</tr>
<tr>
<td>Figure (4):</td>
<td>89</td>
</tr>
<tr>
<td>Patient's categorization based on the degree of asthma control (n: 106).</td>
<td>89</td>
</tr>
<tr>
<td>Figure (5):</td>
<td>92</td>
</tr>
<tr>
<td>Results of skin prick test in studied patients (n = 200 patients).</td>
<td>92</td>
</tr>
<tr>
<td>Figure (6):</td>
<td>93</td>
</tr>
<tr>
<td>Results of SPT to fungal allergens in studied population (n=62 patients).</td>
<td>93</td>
</tr>
<tr>
<td>Figure (7):</td>
<td>93</td>
</tr>
<tr>
<td>SPT to other non-fungal allergens.</td>
<td>93</td>
</tr>
</tbody>
</table>
INTRODUCTION
**Introduction**

Fungi are eukaryotic, unicellular or multicellular, plant-like organisms that do not contain chlorophyll. They are mostly spore-bearing organisms that exist as saprophytes or parasites of animals and plants. Moulds have been defined as a furry growth of microscopic fungi or fungi that produce microscopic reproductive structures. Thus, fungi and moulds are not entirely synonymous, but the terms are often used interchangeably (Volcheck, 2009).

At least 600 species of fungi are in contact with humans and less than 50 are frequently identified and described in epidemiologic studies on indoor environments (Haleem Khan and Mohan Karuppayil, 2012). Colonies of the fungi *Aspergillus fumigatus*, *Alternaria alternata*, *Cladosporium herbarum*, *Penicillium*, and *Fusarium* are universally present in our environment; hence, there is no fungi-free environment (Volcheck, 2009).

Exposure to airborne fungi can occur in both outdoor and indoor environments. Spores are usually present in outdoor air throughout the year, frequently exceeding the pollen population by 100- to 1000-fold or more, depending on environmental factors, such as water, nutrients, temperature, and wind (Latge and Paris, 1991). Spores and fungal fragments found indoors originate from fungi present outdoors and from fungi that might have grown inside the buildings on moist surfaces (Alan et al., 2012).

Fungus has many hazards on human health whether its source is indoor or outdoor as exposure to buildings contaminated with fungi and mycotoxin may cause hypersensitivity pneumonitis (Franks and Galvin, 2010). Exposure to a variety of fungi such as *Aspergillus* and *Fusarium* may result in serious respiratory infections in immune-compromised persons (Jain et al., 2010). In cystic fibrosis or asthmatic patients, *Aspergillus* can cause allergic bronchopulmonary aspergillosis, invasive or semi-invasive pulmonary aspergillosis and pulmonary aspergilloma (Kawel et al., 2011).

Sensitization to fungi is an important factor in patients with allergic respiratory tract diseases, playing a major role in the development, persistence, and severity of lower airway disease, particularly asthma. The major allergic diseases caused by fungi are allergic asthma, allergic rhinitis, allergic sinusitis, bronchopulmonary mycoses, and hypersensitivity pneumonitis (Pieckova and Wilkins, 2004).

The Allergen Nomenclature Sub-committee of the International Union of Immunological Societies (IUIS) had approved the catalog of fungal allergens; this listing includes isoallergens and variants from 25 fungal species belonging to the Ascomycota and Basidiomycota phyla (Nomenclature Subcommittee). Intergenus and
interspecies allergenic cross-reactivity must be distinguished from individual sensitization to multiple fungi (Alan et al., 2012).

Skin prick test (SPT) is used to evaluate sensitization to allergens since this method is considered as easy to perform, inexpensive, convenient to patients, and sufficiently specific and sensitive for the diagnosis of allergen sensitization (Bousquet et al., 2012). Variations in SPT results are dependent on the reagent and manufacturer, potency of extracts, and interpretation of results. The negative predictive result has 95% accuracy. Also major geographic and age variations in the frequency of sensitization to allergens as fungi are observed (O’Driscoll et al., 2009).

To our knowledge, there are deficient data on the prevalent species of moulds and yeast causing skin test reactivity among Egyptian patients with respiratory allergy. The exact prevalence of allergy to moulds and yeast among these patients has never been characterized due to several factors; the use of crude non-standardized allergen extracts for skin testing; the lack of mould spore maps or mould counts; the use of mixtures of different mould species rather than single individual mould species for skin testing.
Aim of The work

The aim of present study is to evaluate the prevalence of sensitization to various species of mould and yeast allergens among other common aeroallergens in Egyptian patients with respiratory allergy.
REVIEW OF LITERATURE
CHAPTER ONE

Allergology origin and allergic response pathogenesis:
Allergology origin and allergic response pathogenesis

Brief history of allergology origin:

Although the term ‘allergy’ was coined in 1906 by Clemens von Pirquet (Von Pirquet, 1906), it was the short lecture given by Paul Portier and Charles Richet on February 15, 1902, at the Societé de Biologie in Paris that is regarded by many authors as representing the birth of modern allergology with the discovery of the phenomenon of anaphylaxis (Portier and Richet, 1902). The phenomenon itself was not totally new. Also it was found as descriptions of similar and sometimes dramatic and untoward reactions in ancient history especially with regard to the famous Pharaoh Menes, who supposedly died in 2641 before century (BC) after being stung by a wasp, hornet or honey bee (Bergmann, 2014).

Today, allergy is defined as a form of atopy or immediate hypersensitivity disease often referring to the type of antigen that elicits the disease. All of these conditions are related to antigen induced mast cell or basophil activation, thus different approaches in diagnosis, therapy and prevention can be taken; so Allergy is not a disease itself, but a mechanism leading to disease (Ring, 2014).

Allergic asthma and rhinitis were already recognized in the 19th century, but the mechanisms behind the diseases were not understood. In 1919, Ramirez noticed that blood transfusion could transfer allergic asthma and passively sensitize the recipient (Blank et al., 2013). In 1921, Prausnitz and Küstner demonstrated passive sensitization of the skin, since then referred to as the PK-test (P for Prausnitz and K for Küstner) (Johansson, 2014). The German physicians Carl Prausnitz and Heinz Küstner performed experiments with themselves, which have become classical today: the injection of Küstner’s serum, who was suffering from a severe fish allergy under the skin of Prausnitz followed by the local application of a fish extract 24 hours later, produced an immediate state of anaphylaxis called cutaneous anaphylaxis (Prausnitz and Küstner, 1921) later it was known that this phenomenon is based on the same mechanisms as the one found by Portier and Richet (Blank et al., 2013).

The search for reagin, the factor in plasma causing the positive PK test, was unsuccessful for about 45 years and some rather confusing proposals were published, e.g. identifying reagin as IgA. In the 1960, Ishizaka et al. published several articles describing an antiserum that could block the PK test indicating that it reacted with reagin. They referred to this antiserum as anti-γE. Not surprisingly considering the very low serum concentration of immunoglobulin E (IgE), they did not succeed in isolating their γE (Ishizaka et al., 1967).
In 1965 Johansson in Uppsala detected in the serum of a myeloma patient an M-component that could not be identified as any of the 4 known immunoglobulin classes. Working with Bennich, the unique immunological and physicochemical characteristics of the new immunoglobulin, provisionally labeled IgND after the initials of the patient, were documented and published. Very small amounts of IgND did, in dose-response, block the PK-test and the active structure was located in the Fc-fragment. A sensitive radio-immuno assay was developed for IgND. Extremely low serum concentrations, in the order of a few nanograms per mL, were found in healthy individuals but, interestingly, 10-100 fold higher levels were found in allergic individuals. Purified IgND was sent to the Ishizakas in 1967 and was found to react with their anti-γE (Johansson, 2014). In February 1968 the World Health Organization (WHO) International Reference Centre in Lausanne, where studies on Ig ND had been performed for some months, invited the two groups to a meeting to review comparative laboratory studies of IgND and γE, resulting in the publication of the official report on the fifth immunoglobulin class, IgE (Bennich, 1968). The discovery of IgE has had a significant impact on the diagnosis and management of allergic disease, enabling clinicians to differentiate between IgE-mediated allergic diseases and other hypersensitivity reactions, and to manage allergic diseases according to their underlying mechanisms. Tests became available that allowed a more simple and reliable diagnosis covering a very broad spectrum of allergens. The characterization and standardization of allergen preparations for clinical diagnosis and allergen specific immunotherapy, ASIT, improved although there is still much to do in this area. An injectable monoclonal anti-IgE is now available that eliminates IgE and has an important role in the management of severe allergic asthma, severe food allergy and chronic urticaria (Johansson, 2014).

**Hypersensitivity reactions classifications:**

The defects or malfunctions in either the innate or adaptive immune response can provoke illness or disease. Such disorders are generally caused by an overactive immune response, known as hypersensitivity reactions; an inappropriate reaction to self, known as autoimmunity; or ineffective immune responses known as immunodeficiency. Hypersensitivity reactions refer to undesirable responses produced by the normal immune system (Warrington et al., 2011).

There are four types of hypersensitivity reactions (Gell and Coombs, 1963):

1. Type I: immediate hypersensitivity or IgE mediated
2. Type II: cytotoxic or antibody-dependent hypersensitivity
3. Type III: immune complex disease
4. Type IV: delayed-type hypersensitivity or T-cell mediated

This original Gell and Coomb's classification categorizes hypersensitivity reactions into four subtypes according to the type of immune response and the effector
mechanism responsible for cell and tissue injury and some diseases have multiple types of immunologic hypersensitivity. Some authors believe this classification system may be too general and favor another classification system proposed by Sell et al. This system divides immunopathologic responses into the following 7 categories:

1. Inactivation/activation antibody reactions
2. Cytotoxic or cytolytic antibody reactions
3. Immune-complex reactions
4. Allergic reactions
5. T-cell cytotoxic reactions
6. Delayed hypersensitivity reactions
7. Granulomatous reactions

This system accounts for the fact that multiple components of the immune system can be involved in various types of hypersensitivity reactions. For example, T cells play an important role in the pathophysiology of allergic reactions. In addition, the term immediate hypersensitivity is somewhat of a misnomer because it does not account for the late-phase reaction or for the chronic allergic inflammation that often occurs with these types of reactions (Sell et al., 1996).

The classification has also been improved so that type IIa is the former type II and type IIb is antibody-mediated cell stimulating (Graves Disease and the "autoimmune" type of chronic idiopathic urticaria). Type IV has four major categories: type IVa is CD4(+)Th1 lymphocyte mediated with activation of macrophages (granuloma formation and type I diabetes mellitus); type IVb is CD4(+)Th2 lymphocyte mediated with eosinophilic involvement (persistent asthma and allergic rhinitis); type IVc is cytotoxic CD8(+) T lymphocyte with involvement of perforin-granzyme B in apoptosis (Stevens Johnson syndrome and toxic epidermal necrolysis); type IVd is T-lymphocyte driven neutrophilic inflammation (pustular psoriasis and acute generalized exanthematous pustulosis). (Uzzaman and Cho, 2012)

**Allergic reactions etiology and pathophesiological mechanism:**

Allergic reactions manifest clinically as anaphylaxis, allergic asthma, urticaria, angioedema, allergic rhinitis, some types of drug reactions, and atopic dermatitis. These reactions tend to be mediated by IgE, which differentiates them from non-IgE-mediated (formerly called anaphylactoid) reactions that involve IgE-independent mast cell and basophil degranulation. Such reactions can be caused by iodinated radiocontrast dye, opiates, or vancomycin and appear similar clinically to urticaria or even anaphylaxis (Lawlor, 1995).
Patients prone to IgE-mediated allergic reactions are said to be atopic. Atopy is the genetic predisposition to make IgE antibodies in response to allergen exposure (Nimmagadda, 1999).

In a person with atopy, exposure of the skin, nose, or airway to a single dose of allergen produces a cutaneous wheal-and-flare reaction, sneezing and runny nose, or wheezing within minutes. Depending on the amount of the allergen, these immediate hypersensitivity reactions are followed by a late-phase reaction, which reaches a peak six to nine hours after exposure to the allergen and then slowly resolves. In the skin, late-phase reactions are characterized by an edematous, red, and slightly indurated swelling; in the nose, by sustained blockage; and in the lung, by further wheezing (Kay, 2001).

Immediate hypersensitivity reactions are mediated by IgE, but T and B cells play important roles in the development of these antibodies. CD4+ T-cells are divided into 3 broad classes: effector T-cells, memory T-cells, and T-regulatory (Treg) cells (Becky et al., 2015).

Effector T-cells are further divided based on the cytokines they produce: TH1, TH2, and TH17 cells. TH1 cells produce interferon-gamma and interleukin (IL)-2, and promote a cell-mediated immune response. TH2 cells produce IL-4 and IL-13, which then act on B-cells to promote the production of antigen-specific IgE. TH17 cells produce IL-17, IL-21, and IL-22 to help fight extracellular pathogens, to produce antimicrobial peptides, and to promote neutrophil inflammation essential for immunity at the skin and mucosal surfaces (Johansson et al., 2002) also abnormalities in the CD4+CD25+FOXP3+ Treg population may play a role in the development of allergic disease (Adkinson, 2009).

The allergic reaction first requires sensitization to a specific allergen and occurs in genetically predisposed individuals. The allergen is either inhaled or ingested and is then processed by an antigen-presenting cell (APC), such as a dendritic cell, macrophage, or B-cell. The antigen-presenting cells then migrate to lymph nodes, where they prime naïve TH cells that bear receptors for the specific antigen (Adkinson, 2009).

After antigen priming, naïve TH cells differentiate into TH1, TH2, or TH17 cells based upon antigen and cytokine signaling. In the case of allergen sensitization, the differentiation of naïve TH cells is skewed toward a TH2 phenotype. These allergen-primed TH2 cells then release interleukin-4 (IL-4), IL-5, IL-9, and IL-13. IL-5 plays a role in eosinophil development, recruitment, and activation. Interleukin-9 (IL-9) plays a regulatory role in mast cells activation. IL-4 and IL-13 act on B cells to promote production of antigen-specific IgE antibodies (Becky et al., 2015).

For this to occur, B cells must also bind to the allergen via allergen-specific receptors. They then internalize and process the antigen and present peptides from it,
bound to the major histocompatibility class II molecules found on B-cell surfaces, to the antigen receptors on TH2 cells. The B cell must also bind to the TH2 cell and does so by binding the CD40 expressed on its surface to the CD40 ligand on the surface of the TH2 cell. Interleukin-4 (IL-4) and IL-13 released by the TH2 cells can then act on the B cell to promote class switching from immunoglobulin M production to antigen-specific IgE production (Becky et al., 2015).

The antigen-specific IgE antibodies can then bind to high-affinity receptors located on the surfaces of mast cells and basophils. Reexposure to the antigen can then result in the antigen binding to and cross-linking the bound IgE antibodies on the mast cells and basophils. This causes the release and formation of chemical mediators from these cells. These mediators include preformed mediators, newly synthesized mediators, and cytokines (Paul, 1999) (Figure 1).

The collective biological activities of these mediators can cause variable clinical responses depending on which organ systems are affected, as follows:

- **Allergic rhinitis:** release of these mediators in the upper respiratory tract can result in sneezing, itching, nasal congestion, rhinorrhea, and itchy or watery eyes.
- **Allergic asthma:** release in the lower respiratory tract can cause bronchoconstriction, mucus production, and inflammation of the airways, resulting in chest tightness, shortness of breath, and wheezing.
- **Urticaria/angioedema:** release in the superficial layers of the skin can cause pruritic wheals with surrounding erythema. If deeper layers of the dermis and subcutaneous tissues are involved, angioedema results. Angioedema is swelling of the affected area; it tends to be painful rather than pruritic.
- **Anaphylaxis:** systemic release resulting in symptoms in 2 or more organ systems is considered anaphylaxis. In addition to the foregoing symptoms, the gastrointestinal system can also be affected with nausea, abdominal cramping, bloating, and diarrhea. Systemic vasodilation and vasopermeability can result in significant hypotension and is referred to as anaphylactic shock (Becky et al., 2015).

These and other cells (eg, monocytes, T cells) are believed to cause the late-phase reactions that can occur hours after antigen exposure and after the signs or symptoms of the acute-phase reaction have resolved. The signs and symptoms of the late-phase reaction can include redness and swelling of the skin, nasal discharge, airway narrowing, sneezing, coughing, and wheezing. These effects can last a few hours and usually resolve within 24-72 hours (Becky et al., 2015).

Finally, continuous or repeated exposure to an allergen (eg, a cat-allergic patient who owns a cat) can result in chronic allergic inflammation. Tissue from sites of chronic allergic inflammation contains eosinophils and T cells (particularly T-helper 2 (TH2) cells). Eosinophils can release many mediators (e.g., major basic protein), which can cause tissue damage and thus increase inflammation. Collectively, this results in structural and functional changes to the affected tissue. Furthermore, a repeated allergen challenge can result in increased levels of antigen-specific IgE, which ultimately can
cause further release of interleukin-4 (IL-4) and interleukin-13 (IL-13), thus increasing the propensity for TH2 cell/IgE–mediated responses (Paul, 1999).

Figure 1: illustrate Pathways Leading to Acute and Chronic Allergic Reactions (Kay, 2001)
CHAPTER TWO

Epidemiology
Epidemiology

Global problem in children and adults

Allergies are increasing dramatically worldwide. Approximately 10% to 30% of the world’s adult population and up to 40% of children are affected by some form of allergy (Figure 2).

Respiratory allergies are the most common allergies in Europe and worldwide. Allergic rhinitis (with or without conjunctivitis) affects 5% to 50% of the population worldwide among which 15% to 20% suffer from a severe form of the disease (White, 1998), and its prevalence is increasing (Bousquet, 2001). It is estimated that allergic asthma affects 5% to 12% of people in Europe (Masoli, 2004).

It is not an easy task to compare prevalence data from different European countries. In fact, there is no clear common definition of the disease for prevalence purposes. In most cases, if official data are available, the definition of allergy doesn’t always include all allergic reactions. Moreover, in some countries, statistics may report only severe cases of hospitalization, and exclude mild/moderate allergies, or patients who only use over-the-counter medicines. Similarly, differences in prevalence may also depend on the degree of awareness about allergic diseases (White, 1998).

Figure 2: Prevalence of allergies (not only respiratory allergies) in the countries surveyed (Valovirta, 2001).
In children, asthma is the most common chronic disease in childhood and the leading cause of childhood morbidity from chronic disease as measured by school absences, emergency department visits and hospitalizations. Allergen-specific sensitization is one of the most important risk factors for the development of asthma in children according to 2017 GINA Report, Global Strategy for Asthma Management and Prevention. In addition, children with one form of allergy are more likely to develop other forms of allergy. For instance, at a very young age they may have food allergies, and as this improves they develop respiratory allergies. The progression of one manifestation of allergy to another over a period of time is known as the “allergic march”. Therefore, early diagnosis and adequate control of allergic rhinitis is crucial to halt the progression of the disease to asthma (Kulig et al., 1999).

The WAO (World Allergy Organization) White Book on Allergy, published in 2011 by the World Allergy Organization confirms that the prevalence of allergic rhinoconjunctivitis and allergic asthma is increasing worldwide. Allergic rhinoconjunctivitis is the most common non-infectious rhinitis (Pawankar et al., 2011).

Many studies have been performed to understand the epidemiology of respiratory allergies (allergic rhinoconjunctivitis and allergic asthma) in different countries. For example, the International Study of Asthma and Allergies in Childhood (ISAAC), which involves 306 centres in 105 countries, was established in 1991 to investigate asthma, rhinoconjunctivitis and eczema in children due to considerable concern that these conditions were increasing worldwide. As suggested by the International Primary Care Respiratory Group (IPCRG) in the introduction to the WAO White Book, differences in prevalence among countries could be due to underreporting or to a lack of awareness of these diseases in deference to more important socioeconomic medical problems (Lai, 2009).

### Prevalence of allergic rhinitis worldwide

In a study of over 9000 people in Europe, Bauchau et al. found that the prevalence of subjects with clinically confirmable allergic rhinitis ranged from 17% in Italy to 29% in Belgium, and the overall prevalence was 23%. But, surprisingly, 45% of these subjects had not been previously diagnosed by a physician. These statistics confirm the high prevalence of allergic rhinitis in Western Europe and demonstrates that this condition is frequently undiagnosed (Bauchau et al., 2004).

The data from the countries surveyed confirm the prevalence reported in the study by Bauchau et al. The severity of allergic rhinitis symptoms is not considered in official statistics. The symptoms of allergic rhinitis are distressing and negatively impact on the
patient’s quality of life. Since allergic rhinitis is such a “neglected” condition, many patients who would benefit from treatment fail to receive it (Bauchau et al., 2004).

**Prevalence of asthma worldwide:**

In most of the countries surveyed there are no national statistics for allergic asthma alone, therefore table 2 report data for all types of asthma (Table 1).

Nevertheless, it must be noted that an allergy is the cause of asthma in about 80% of cases. Furthermore, according to the WAO, about 50% of asthmatics older than 30 years of age are concomitantly allergic. Younger asthmatics have an even higher incidence of allergies (Li, 2015).

Studies from Europe and USA indicate that one-third of school age children with asthma may be undiagnosed (Pawankar, 2011). Asthma is frequently undiagnosed also in adults and particularly in the elderly (Valovirta, 2001).

In most patients with a diagnosis, asthma may not be controlled. This is partly because physicians often fail to appreciate the severity of their patient’s asthma, and partly because patients do not take their prescribed controller medication (Pawankar, 2011).

In addition, follow-up visits are not planned in advance; and often patients seek medical advice only when they have an acute asthma exacerbation. This worrying information illustrates the need for increased awareness and education about asthma among physicians, patients and their families, as well as policy makers. In Ireland, the HARP (Helping Asthma in Real Patients) study, conducted in conjunction with the IPCRG (International Primary Care Respiratory Group), the Asthma Society of Ireland and the Irish Association of General Practitioners found that the asthma was uncontrolled in 60% of patients and over 50% of respondents reported symptoms of mild rhinitis with a further 20% reporting symptoms of significant rhinitis. In addition, respondents with uncontrolled asthma were more likely to have significant rhinitis (25%), and more likely to have symptoms of rhinitis (12%) than respondents with controlled asthma (15% and 27% respectively) (Valovirta, 2001).
<table>
<thead>
<tr>
<th>Country</th>
<th>Prevalence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>4.3%</td>
<td>Statistik Austria (2006/2007)</td>
</tr>
<tr>
<td>Bulgaria</td>
<td>9%</td>
<td>Allergy and asthma Suppl, 2000 and Official Reports of President of Society, 2006, 2007-2010</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>8%</td>
<td>Kratenova J. National Institute of Public Health</td>
</tr>
<tr>
<td>Denmark</td>
<td>6.4%</td>
<td>Danish Institute of National Statistics</td>
</tr>
<tr>
<td>Finland</td>
<td>Adults 8-10%</td>
<td>Finnish Allergy Programme 2008 -2018</td>
</tr>
<tr>
<td></td>
<td>Children 5%</td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>6.7%</td>
<td>IRDES (Institut de Recherche et Documentation en Economie de la Santé, the national health economics research institute) survey - n. 138 Dec 2008</td>
</tr>
<tr>
<td>Ireland</td>
<td>11%</td>
<td>Elaborated from ISAAC (International Study of Asthma and Allergies in Childhood) and Central Statistics Office</td>
</tr>
<tr>
<td>Norway</td>
<td>9% in Adults</td>
<td>Long term trends in asthma in Oslo, Norway: survey methods, symptoms and diagnosis, Jan Brogger, Doctoral dissertation 2004</td>
</tr>
<tr>
<td>Sweden</td>
<td>10%</td>
<td>The Swedish National Institute of Public Health – 2010</td>
</tr>
<tr>
<td>Switzerland</td>
<td>2.3%</td>
<td>Global Initiative for Asthma (GINA) 2004</td>
</tr>
</tbody>
</table>

Table 1: Prevalence of asthma worldwide (Valovirta, 2001).
Egypt prevalence of respiratory allergic diseases:

Few studies evaluated asthma prevalence in Egypt. In a survey including 115 health centers in five governorates, Khallaf et al. reported that asthma prevalence was 4.8% in Egypt (Khallaf et al., 1993) then El-Hefny found that asthma prevalence was 8.2%, using a questionnaire among 13028 children 3-15 years old (El-Hefny, 1994).

Georgy et al. used translated and adapted version of the ISAAC (International Study of Asthma and Allergies in Childhood) questionnaire which was distributed to a sample of 2645, 11-15 years old school children in Cairo. They revealed that wheeze during 2006 was 14.7% and physician diagnosed asthma was 9.4% (Georgy et al., 2006).

In Alameldin et al. study, the prevalence of questionnaire-diagnosed asthma was in Assiut district in Upper Egypt. It was a cross sectional study that conducted among preparatory school students in Assiut city and two rural areas in Assiut district. Twelve schools were selected randomly from different regions in Assiut city and two rural areas one to the North and the other to the South of Assiut city. The total coverage of the students included was 1048 (482 boys and 566 girls). Data were collected by self-administered questionnaire (in Arabic Language) which was filled by the participants. Of the 1048 positively responding subjects, 65 fitted the diagnosis of asthma with overall prevalence of 6.2% (Alameldin et al., 2012). This rate is less than what has been previously estimated in Cairo in 2006 by Georgy et al., This may be due to the different geographical, social and environmental factors between these two localities. According to Ministry of state for environmental affairs, Egyptian environmental affairs agency, Air Quality reports in Cairo, 2000-2001, Cairo is mainly an urban district surrounded by multiple industrialized areas where air pollution by sulphur dioxide, nitrogen dioxide, carbon monoxide, ozone and particulate matter is one of the highest levels worldwide due to industrialization and heavy traffic, while Assiut is a semiurban area surrounded by rural societies, and has less industrial areas (Hassan and Hagrass, 2017). Also Alameldin et al. concluded that, although most asthmatic children were males (55.4%), the prevalence of asthma among studied children was not significantly associated with gender (Alameldin et al., 2012).

Ali et al. concluded in 2014 the prevalence of asthma was 9.1%, and commonly reported among males than females. The study was to determine the prevalence of bronchial asthma and to investigate its impact on the cognitive functions and academic achievement among preparatory school children in Damietta Governorate, north of Cairo (Ali et al., 2014). The results revealed that asthma was more prevalent among students living in the lowest economic levels and with high crowding index. The consanguinity among parents, positive family history of asthma, passive smoking, presence of other allergies, contact with birds, contact with animals, presence of cockroaches, and frequent chest infection early in life were risk factors which were significantly associated with asthma. The asthmatic attacks were commonly reported in
the winter and were reached to its peak at night. The bronchial asthma has a negative impact on cognitive abilities and academic achievement (Ali et al., 2014).

In Alameldin et al. study there was no significant difference regarding the prevalence of childhood asthma in urban and rural areas that may be explained by the similarity in environmental conditions in both areas due to their close proximity to each other and relatively low levels of air pollutions in Assiut as it is a semiurban area surrounded by rural societies with few numbers of factories and industries. Downs et al. also did not find a protective effect of farming among children living in a primary crop farming region (Downs et al., 2001). In contrast, a cross sectional survey of children in Austria, Germany, and Switzerland revealed a decrease in prevalence of asthma, hay fever, and atopic sensitization among children living in farms (Riedler et al., 2001).
CHAPTER THREE

Respiratory allergic diseases
Respiratory allergic diseases

One airway, one disease.

Is it a link between allergic rhinitis and allergic asthma?

The important link between the upper and lower airways has been highlighted in the Allergic Rhinitis and Its Impact on Asthma (ARIA) guidelines. Developed in 1999, the ARIA guidelines replaced the International Consensus on Rhinitis (IRC) guidelines of 1994 and offer the advantage of being evidence based. The concept of “one airway, one disease” was highlighted in the Allergic Rhinitis and Its Impact on Asthma guidelines and had arisen as a result of the now well-established link between the upper and lower airways (Durham, 2002).

Allergic rhinitis should be considered as a risk factor for asthma along with other known risk factor. Patients with persistent allergic rhinitis should be evaluated for asthma by means of a medical history, chest examination, and, if possible and when necessary, the assessment of airflow obstruction before and after bronchodilator. Patients with asthma should be appropriately evaluated (history and physical examination) for rhinitis. Ideally, a combined strategy should be used to treat the upper and lower airway diseases to optimize efficacy and safety (Bousquet et al., 2008).

It has even been proposed that allergic rhinitis and allergic asthma are manifestations of the same disease entity and that they represent a continuum of disease. In this way, subjects with less severe disease express only rhinitis, while subjects with more severe disease express both rhinitis and asthma. This concept has been labeled “one airway, one disease” (Grossman, 1996), “allergic rhinobronchitis” (Simons, 1999), or “united airways disease” (Passalacqua et al., 2000). So According to this concept, allergic rhinitis should be ubiquitously present in subjects with allergic asthma (Linneberg et al., 2002).

The association between allergic rhinitis and allergic asthma appears to be stronger than has been reported by authors Grossman, 1996; Simons, 1999 and Passalacqua et al., 2000. A possible explanation could be that the definitions of allergic rhinitis and allergic asthma used in the recent studies were very specific. Kapsali et al., however, reported a high prevalence of 98.9% (360/364) of allergic rhinitis in patients with allergic asthma (Kapsali et al., 1997), whereas Greisner et al. reported that allergic rhinitis was present in 85.7% (72/84) of asthmatics in a follow-up study of college students (Greisner et al., 1998).
The relationship between rhinitis and asthma symptoms was stronger for animal and mite allergy than for pollen allergy. Thus, approximately one-quarter of subjects with allergic rhinitis to pollen had in addition allergic asthma to pollen, while approximately one-half of subjects with allergic rhinitis to animals (or mite) had in addition allergic asthma to animals (or mite). Moreover, the risk of developing allergic asthma appeared to be higher for allergic rhinitis related to perennial allergens than allergic rhinitis related to seasonal allergens. The reason for this is not clear (Linneberg et al., 2002).

The Copenhagen Allergy Study conducted by Linneberg et al. had demonstrated a remarkably strong epidemiologic association between rhinitis and asthma when both relate to inhalant allergy, a finding that suggests that allergen driven IgE-mediated inflammation of the airways may represent the pathophysiologic link between these manifestations (Linneberg et al., 2002). On the other hand, a strong association between nonallergic rhinitis and nonallergic asthma has also been demonstrated (Leynaert et al., 1999).

Allergic rhinitis and allergic asthma may also be linked from a therapeutic point of view. Thus, it is of interest that pharmacological treatment of the nose tends to improve coexisting asthma (Corren, 1998). Furthermore, studies of allergen specific immunotherapy in patients with allergic rhinitis due to pollen allergy have found improvements of coexisting asthma (Bousquet et al., 1998). Other authors have hypothesized that allergen-specific immunotherapy prevents the development of asthma in children with allergic rhinitis due to pollen allergy (Valovirata, 1997) and in adolescents with allergic rhinitis due to mite allergy (Grembiale, 2000).

Also Van Den Nieuwenhof et al., 2009 study found that physician diagnosed allergic rhinitis (AR) is an independent risk factor for a future diagnosis of asthma with increasing the risk almost fivefold for an asthma diagnosis later in life (van den Nieuwenhof et al., 2009).

The Bidirectional Link between the Nose & the Bronchi: experimental evidences.

The support for the connection between AR and asthma has been given impetus with a series of elegant studies by Braunstahl et al on tissue response to allergen provocation. These studies were designed to elucidate the pathophysiological connections between the nose and the lungs (Braunstahl et al., 2000).

The first study compared allergic inflammation and clinical findings from the upper and lower airways after segmental bronchial provocation (SBP) with an allergen. Baseline nasal and bronchial specimens were collected from patients and controls before and after SBP. The allergic inflammatory response was determined by comparing the specimens at baseline, and 1 hour and 24 hours after SBP (bronchial biopsy only
after 24 h). Signs and symptoms were recorded for each time point. The specimens were examined for mucosal allergic inflammation, and evaluated for the presence of eosinophils, IL-5+ cells and eotaxin+ cells (necessary for eosinophil survival and chemotaxis). The study selected eight AR patients (with symptoms and skin-prick test confirmation) and eight nonallergic, healthy controls (Braunstahl et al., 2000). Baseline tissue staining revealed greater numbers of eosinophils (tissue indicators for an allergic response) in allergic patients. Although the allergen was specifically directed to the lungs, both nasal and bronchial tissue exhibited significant increases in cells of the inflammatory cascade. These results suggested a general systemic activation of eosinophils and migration to the mucosa of both the upper and lower airways, i.e., a shared inflammatory response even with exposure only in bronchial tissue (Braunstahl et al., 2000).

The second study had a similar provocation except that this time, instead of the bronchi, the nasal passages were stimulated (Braunstahl et al., 2001). Two groups of patients, nine with AR and nine nonallergic, healthy controls, were selected for the study. Blood samples, as well as bronchial and nasal biopsy specimens, were collected from the participants before nasal provocation and 24 hours after provocation. Other measures included nasal and bronchial symptom scores (visual analogue scale), peak nasal inspiratory flow (PNIF) and peak expiratory flow (PEF; lung function measures). These measures were examined at baseline, after 0.5 hour and every 2 hours for 12 hours, and at day 2. At baseline, the subjects in the study were comparable, but after nasal provocation, the AR patients had lower values of PEF, and the PNIF revealed a bimodal curve of reduced air flow. Minimal changes were seen in the control subjects. In AR subjects, symptoms and the PNIF returned to baseline after 24 h, but the PEF continued to show a reduced air flow (Braunstahl et al., 2001).

Taken together, results suggest that allergen challenge in any part of the airways leads to differentiation and migration of progenitors from the bone marrow, and directly or indirectly affects both upper and lower airway function (Kim et al., 2008).

The respiratory tract can be considered a single morpho-functional entity (Undem et al., 1999). It is lined, until the bronchi of the lower dimension, by a ciliated epithelium and mucinous glands with a dense network of vascularization and innervation. The latter, in particular, appears very similar in the upper and lower compartments, supporting the hypothesis of the so-called nose (sinu)–bronchial reflex as a possible pathogenetic mechanism (Enrico et al., 2010). Although this phenomenon has been extensively observed in animals, some concerns remain regarding its existence in humans, despite the fact that it has been shown that nasal challenge is able to stimulate a contractile bronchial response or increase bronchial responsiveness (Littell et al., 1990).
The respiratory mucosa is rich in mast cells capable of contributing to nose–bronchi connections through the release of mediators and cytokines (Okuda, 1999). Lymphoid tissue forms the mucosal-associated lymphoid tissue (MALT) and is widely represented in the nose and bronchi. The major difference between the two compartments is that the nasal mucosa is located in a rigid structure and contains sinusoid vessels with erectile features, whereas the bronchi are contained in an elastic parenchyma, rich in peribronchial smooth muscle (Gunst and Tang, 2000). This anatomical diversity explains the different responses to trigger stimuli: rhinorrhoea and obstruction in the nose, and secretions and bronchoconstriction in the lung. It is known that the upper respiratory tract acts as a physical filter, sounding board, heater and humidifier of inhaled air, so it is easy to assume how the impairment of any of these functions results in an alteration of the lower-tract homeostasis. There is evidence that the hyperventilation of cold air through the mouth in asthmatics facilitates the reduction of parameters of respiratory function and increases nasal resistance (McLane et al., 2000).

Another potential functional link between the nose and bronchi has been identified in the systemic impact of local inflammation via soluble mediators that involve the bone marrow (Denburgh, 1999). This latter responds in a rapid and specific way to nasal challenge, with an increased production of precursors and mature eosinophils (Gaspar et al., 1997).

Support for the inflammatory link between the upper and lower airways was found in a study that included 14 children with AR and 15 with AR and asthma. Basal sputum eosinophil cationic protein (ECP) and exhaled nitric oxide (eNO) values before allergen-specific nasal challenge were significantly higher in both groups compared with healthy controls. The increase in sputum ECP and eNO after allergen-specific nasal challenge in rhinitic children without wheezing could suggest the use of these mediators as positive predictive markers of evolution towards asthma (Marcucci et al., 2007).

Studies often examined the prevalence and pathogenic roles of Th1/Th2, or Th17/Treg balance in children with allergic asthma or rhinitis alone (Albano et al., 2013). However, rare studies were seen focusing on this balance in children with both BA and AR. In Baohong et al., study, results demonstrate that the imbalance of peripheral Th17/Treg cells plays an important role in the pathogenesis of AR accompanying with BA. In this study, the results showed for the first time that the proportion of peripheral Th17 cells in AR accompanying with BA was significantly higher than those of children with AR or BA alone and the controls, accompanied by an increase in the expression of ROR-γt mRNA and serum levels of IL-6, IL-17 and IL-23. Additionally, the proportion of Treg cells in peripheral blood of AR accompanying with BA children was significantly lower than that of children with AR or BA alone and the controls, accompanied by the reduction of serum Transforming growth factor beta 1 (TGF-β1) level. Furthermore, the ratio of Th17/Treg cells in peripheral blood and ROR-
γt/Foxp3 mRNA expression were also significantly higher in children with AR accompanying with BA than in children with AR or BA alone. These findings suggest that Th17/Treg imbalance plays an important role in the pathogenesis of AR accompanying with BA (Tao et al., 2015).

**Effects of Treatment on Rhinitis & Asthma**

It has been shown that treatment with nasal steroids, combined with inhaled steroids, is able to reduce BHR to methacoline challenge in subjects with asthma and to improve symptoms by reducing visits to the emergency department for exacerbation (Durham, 1999).

A Cochrane review performed a meta-analysis of 14 studies and found a trend suggesting that intranasal corticosteroids improve asthma symptoms and FEV₁, but the result was not found to be statistically significant (Taramarcaz and Gibson, 2003). Later, 863 patients in an open randomized trial with persistent asthma treated with inhaled steroids, plus the addition of antileukotrienes or nasal steroids for the treatment of seasonal AR, experienced no additional improvements in overall asthma control compared with inhaled steroids alone (Nathan et al., 2005).

In other circumstances, the failure to consider the treatment of rhinitis as essential to asthma management impaired clinical control of asthma, suggesting that asthma and rhinitis, in some patients, can be controlled by the exclusive use of nasal medication (Stelmach et al., 2005).

Curiously, the hypothesis that nasal inhalation of a corticosteroid can be effective both for rhinitis and asthma was examined with positive findings in several studies where budesonide had been nasally administered through a special spacer, resulting in both upper and lower airways deposition (Pedersen et al., 1998). Alternatively, exhaling a budesonide inhaler through the nose results in a significant reduction in dose requirement of budesonide nasal spray in patients who have asthma with rhinitis (Shaikh, 1998).

Another study examined the effects of orally inhaled budesonide (avoiding nasal deposition of the drug) on nasal allergic disease in seasonal AR without asthma and found a reduction of nasal and circulating eosinophilia with an attenuation of seasonal nasal symptoms. The treatment of AR with triamcinolone resulted in a decrease of two noninvasive markers of lower airway inflammation, eNO and H₂O₂, supporting the concept that upper and lower airway inflammation should be seen as a continuum (Sandrini et al., 2003).
Conversely, treatment of the pulmonary compartment had a measurable impact on measures of nasal allergic inflammation (i.e., symptoms scores, eosinophil numbers and ECP levels in nasal lavage fluid). Bronchial budesonide inhibits the seasonal increase in nasal and blood eosinophils, and nasal lavage levels of Eosinophil cationic protein (ECP). These observations should be kept in consideration, as the distant effects after local treatment may be caused by systemic medication absorption (Grieff, 1998).

Antihistamines at therapeutic doses do not affect asthma, but their beneficial action on the upper airway contributes to improve the overall management of the 'one airway disease (Mirsadraee et al., 2004).

A protective effect of antihistamines on BHR after nasal allergen challenge in patients with AR can be explained either by a direct effect of the drug on the lower airways or, indirectly, by protection related to the control of rhinitis (Aubier, 2001). An effect on systemic inflammation and on nasal and bronchial mucosal inflammation after nasal allergen provocation in subjects with grass-pollen AR and asthma has been reported (Reinartz et al., 2005).

During the pollen season, antihistamine treatment reduces the symptoms of asthma, although concerns exist regarding differences in lung function (Baena Cagnani et al., 2003). The synergistic effect with drugs of other classes (antileukotrienes, α-mimetics) may reduce the use of bronchodilators and improve peak flow in mild asthma (Corren, 1997). In children, the occurrence of respiratory infections and exacerbations of asthma may be reduced by continuous antihistamine treatment (Ciprandi et al., 1999).

The impact of antileukotriene (leukotriene receptor antagonist [LTRA]) treatment in patients with both AR and asthma has also been evaluated. Since the pathophysiology of the two diseases involves the inflammatory cascade and eosinophil influx into nasal and bronchial mucosae, these drugs are expected to improve both the conditions. Observational studies and clinical trials showed that LTRAs are effective in treating both the upper and lower airways in patients with asthma and AR (Wilson et al., 2004).

The apparent success of specific immunotherapy in children with AR in preventing the development of asthma and bronchial hyper-responsive (BHR), seen in many longitudinal studies, provides a powerful argument supporting a common pathogenetic mechanism in allergic respiratory disease (Moller et al., 2002). Compared with placebo, short-term co-seasonal sublingual immunotherapy resulted in lower asthma symptoms and reduced use of medication in the second and third years of therapy, associated with a reduction in asthma development. Similar findings have been reported on the effect of immunotherapy on the degree of nonspecific BHR in patients with allergic bronchial asthma and/or AR (Novembre et al., 2004).
Various studies have provided evidence that treatment of AR with specific immunotherapy, either sublingual or subcutaneous, can reduce bronchial hyper-responsive (BHR) and may prevent asthma, but further investigation is needed to confirm the preventative role of specific immunotherapy. The potential of an early intervention with the aim to change the natural history of allergic disease remains both attractive and promising (Compalati et al., 2009).

In the study on effects of immunotherapy in allergic rhinitis individuals with bronchial hyper-responsive (BHR) by Grembiale et al., there was a 4-fold increase in the provocative dose of metacholine in the bronchial provocation test after 2 year of immunotherapy with house dust mite in the immunotherapy group in a double blind placebo controlled setting. There was no change in the placebo group (Grembiale et al., 2000).

In the study by Jacobsen et al the results of 36 adult patients receiving immunotherapy with standardized tree pollen extracts for 3 years show after the period of 6 years that none of the rhinitis patients developed asthma during the study period, i.e., during 9 years (Jacobsen et al., 1997).

To answer the question” Does allergen specific injection immunotherapy stop the development of asthma in children suffering from allergic rhinoconjunctivitis induced by birch and/or timothy pollen allergy?” The Preventive Allergy Treatment (PAT) Study has been started in 1992 in children aged from 7 to 13 years. The results show that children treated actively with the standardized birch and/or timothy pollen extracts had significantly fewer asthma symptoms after 3 years of treatment compared to the control group as evaluated by clinical diagnosis. In the active group, also the methacholine bronchial provocation test results improved significantly (Möller et al., 2002).

It is documented in several studies that injection immunotherapy is acting by influencing basic immunological mechanisms. It is possible that when immunotherapy improves the symptoms from one part of the airway i.e., the nose, it also has the potential to give the same immunologic response in another part, i.e., lungs (Ebner et al., 1997).
Bronchial asthma:

Definitions

Asthma is a common, chronic respiratory disease affecting 1 to 18% of the population in different countries. According to 2017 GINA Report, Global Strategy for Asthma Management and Prevention, Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation.

Additionally, Lehmann and Williams have come to appreciate that asthma is a highly complex disease and despite some major advances, it is still not fully known the cross-talk that is involved between the external environment, the pulmonary immune system, and gene–environment interactions that predispose certain susceptible individuals to the allergic asthmatic condition (Dietert and Luebke, 2012).

Several international groups have defined asthma broadly and provided recommendations to treat disease physiology e.g., forced expiratory volume in the 1st second (FEV1). Expert Panel Report 3—National Asthma Education and Prevention Program: “Asthma is a common chronic disorder of the airways that is complex and characterized by variable and recurring symptoms, airflow obstruction, bronchial hyperresponsiveness, and an underlying inflammation.” Classified clinically as Intermittent or Persistent (Mild, Moderate, Severe) (Amir et al., 2012).

Asthma phenotypes versus endotypes:

Variability in clinical characteristics, inflammatory profiles and responses to treatment has made it increasingly clear that severe asthma is not a single disease. Treating asthma based on phenotypes has been shown to be suboptimal. Although phenotyping refers to grouping individuals with similar observable characteristics, endophenotyping, or ‘endotyping’ for short, groups individuals on the basis of underlying molecular mechanisms or treatment responses (Lotvall et al., 2011).

Patient phenotypes and subphenotypes (or clusters) exist that confound treatment goals, as indicated by the recognition that poor responses to inhaled corticosteroids (ICS) are not uncommon. Some argue that while the atopic, Th2 paradigm of immune dysregulation is useful, it does not encapsulate the incredible heterogeneity in asthma, thus calling for a re-evaluation of how we conduct clinical trials. Instead, subtypes of asthma may be defined functionally or pathologically by specific molecular mechanisms or by a distinct treatment response (Anderson, 2008).
Five distinct clinical subphenotypes or “clusters” of asthma have been described, possibly representing different pathophysiologic mechanisms, thus creating an opportunity to develop individualized novel therapies (Moore et al., 2010).

The Severe Asthma Research Program (SARP) has recently defined five distinct clinical asthma subphenotypes (Moore et al., 2010) that will hopefully change future research by providing more homogenous cohorts to study. Cluster analysis was used to identify five groups or clusters of asthmatics, where age ≥12 years old were considered “late onset”: cluster 1 includes those with early-onset atopic asthma, normal lung function, on two or fewer controller medications, and have minimal health care utilization. Cluster 2 consists of subjects with early-onset atopic asthma and preserved lung function, but increased medication requirements and health care utilization. Cluster 3 is composed of mostly older obese women with late-onset non-atopic asthma, moderate reductions in FEV1, and frequent oral corticosteroid use to manage exacerbations. Cluster 4 includes asthmatics with severe airflow obstruction and bronchodilator responsiveness but who differ in to their ability to attain normal lung function, and have a very early age of asthma onset in childhood (not adolescence), atopic status, and use of oral corticosteroids. Cluster 5 is similar to cluster 4, but consists of more women (63%) with later-onset disease (69% late onset), less atopy (66%), and longer duration of disease (Moore et al., 2010).

According to 2017 GINA Report, Global Strategy for Asthma Management and Prevention, many phenotypes have been identified. Some of the most common include:

• **Allergic asthma:** this is the most easily recognized asthma phenotype, which often commences in childhood and is associated with a past and/or family history of allergic disease such as eczema, allergic rhinitis, or food or drug allergy. Examination of the induced sputum of these patients before treatment often reveals eosinophilic airway inflammation. Patients with this asthma phenotype usually respond well to inhaled corticosteroid (ICS) treatment (Bel 2004, Wenzel 2012).

• **Non-allergic asthma:** some adults have asthma that is not associated with allergy. The cellular profile of the sputum of these patients may be neutrophilic, eosinophilic or contain only a few inflammatory cells (paucigranulocytic). Patients with non-allergic asthma often respond less well to ICS.

• **Late-onset asthma:** some adults, particularly women, present with asthma for the first time in adult life. These patients tend to be non-allergic, and often require higher doses of ICS or are relatively refractory to corticosteroid treatment.

• **Asthma with fixed airflow limitation:** some patients with long-standing asthma develop fixed airflow limitation that is thought to be due to airway wall remodeling.
• Asthma with obesity: some obese patients with asthma have prominent respiratory symptoms and little eosinophilic airway inflammation (Bel 2004, Wenzel 2012).

Although some overlap does exist among these clusters, the divergent phenotypic characteristics described in this analysis suggest disease mechanisms very different from atopy and immunoglobulin E (IgE)-mediated inflammation (i.e., Th2 immune responses) which may determine the variable clinical responses. It is of great interest to ascertain whether specific biomarkers for each asthma cluster exist to promote more effective diagnosis and treatment (Amir et al., 2012).

Genetic and Immunological insights:

The immunological basis of asthma and the role of Th1 and Th2 immune pathways is well-established (figure 3). The IL-17 producing T cells and regulatory T cell (Treg) types have recently emerged as key players in asthma pathogenesis. It is generally accepted that Th1 cells antagonize the effects of Th2 cells in asthma, and vice versa. Similarly, Treg cells (implicated in immune tolerance and autoimmunity) also inhibit Th2 cell responses in asthma. The Th2 cytokines IL-4 and IL-13 are important in both human and animal models of allergic asthma. Aeroallergen-induced activation of airway dendritic cells, leads to subsequent B and T cell activation, which in turn leads to recruitment of mast cells, eosinophils, macrophages, and neutrophils. Ultimately, these same processes and increased production of proinflammatory chemokines and cytokines promote airway remodeling in various resident cell types (Amir et al., 2012).

Beyond adaptive immune responses to allergens (i.e., antigen-dependent), the innate immune response which is antigen-independent has emerged as a major mechanism in asthma pathogenesis and is an area of burgeoning research (Amir et al., 2012).

The cytokine profiles of the bronchial mucosa in allergic and intrinsic asthma are distinctly different. Expression of the interleukin-4 (IL-4) receptor is lower in intrinsic asthmatics and is associated with decreased expression of the transcription factor STAT6 (Christodouloupolos et al., 2001). Also, BAL fluid collected from intrinsic asthmatics contains higher levels of RANTES (i.e., CCL5) than BAL fluid collected from allergic asthmatics (Ying et al., 1999). RANTES is produced by macrophage subtypes (Ying et al., 1999) and may be involved in eosinophilia (Novak and Bieber, 2003).

Intrinsic asthma is also associated with an increase in the abundance of macrophages in the bronchial mucosa expressing the alpha subunit of the GM-CSF receptor (Kotsimbos et al., 1997). The elevated number of macrophages has been hypothesized to be due to (1) recruitment of a subset of activated macrophages into the airway, (2) macrophage dysfunction, or (3) an ongoing infectious or autoimmune disease. Despite
the clinical differences between allergic and intrinsic asthma, the basic similarities have led some to argue that these are not distinct entities immunopathologically (Humbert et al., 1999).

It is generally believed that airway remodeling is the consequence of Th2-mediated chronic inflammation (Murdoch and Lloyd, 2010). Th2 cells that secrete IL-4, IL-5, and IL-13 infiltrate the asthmatic airway leading to eosinophilia, leukocytosis, and subsequent activation of mast cells and IgE secretion from B cells (Loza and Chang, 2007).

In atopic individuals, IgE concentrations can be up to 1,000 times greater than in normal individuals but remain at levels that are still far lower than the normal baseline concentrations of other immunoglobulin isotypes. CD4+ Th2 cells play a crucial part in this isotype class switching to IgE and enhanced IgE production. Th2 cells are the primary sources of cytokines such as IL-4 and IL-13 required for switching immunoglobulin synthesis to the IgE isotype. However, natural killer T (NKT) cells, mast cells, or basophils and eosinophils can also secrete IL-4 and IL-13 though NK cells can only produce IL-13 (Dietert and Luebke, 2012).

Pollen, pet dander, and excreta from house dust mites and cockroaches are common atopy-inducing allergens. The classic signs of atopy are elevated levels of allergen-specific IgE responses and positive skin prick tests to common allergens (Willart and Lambrecht, 2009). Antigens that provoke atopy are typically soluble proteins often complexed with indoor or outdoor airborne particulate matter that is both respirable and migratory to the upper and lower airways of those individuals that come into contact with these materials. Thus, atopy is a predisposition of an individual to develop an IgE-mediated disease that is largely characterized by an eosinophilic infiltration (Dietert and Luebke, 2012).
Hygiene hypothesis

The original ‘hygiene hypothesis’ was prompted by evidence that overcrowding, unhygienic conditions and also larger family size were associated with a lower prevalence of allergic diseases as eczema, hay fever and asthma (Asher et al., 1998 and Kramer et al., 1999). Increased infections and exposures to microorganisms, particularly early in life, have been proposed as an explanation for these findings (Sly et al., 2008, Flohr et al., 2006) (figure 4).

However, there is a considerable body of evidence warranting scepticism about the hygiene hypothesis as the primary explanation for global asthma prevalence time trends in particular (Brooks et al., 2013). First, it has now well been established that the proportion of asthma attributable to atopy is usually less than one-half, with a similar proportion attributable to eosinophilic airway inflammation, the hallmark of allergic asthma (Douwes et al., 2002). The original hygiene hypothesis suggests that decreased microbial exposure would, through enhanced atopic immune responses, increase the incidence of allergies and allergic asthma. If true, then the protective effects would be
most pronounced for the atopic asthma phenotype as well as other atopic conditions such as eczema. However, there is some evidence that nonatopic asthma may have increased more than atopic asthma (Thomsen et al., 2004). Also, farming exposures may also be protective against non atopic wheeze, suggesting that microbial exposure may not be only affecting the prevalence of allergic conditions. Furthermore, in a repeated population survey among preschool children, an increase in asthma prevalence was not only found in children with the classic asthma pattern of wheeze but in all wheezing phenotypes including viral-induced wheezing (Kuehni et al., 2001 and Brooks et al., 2013).

A second anomaly is the high asthma prevalences in Latin America, which appear unlikely to have lower infection rates than European countries such as Spain and Portugal which have lower asthma symptom prevalence (Pearce and Douwes, 2006). Despite this, the early epidemiological findings showing that daycare attendance and farm animal contact were protective of atopy and asthma have been replicated in Latin America (Chile), suggesting a similar effect to that originally observed (Boneberger et al., 2011).

Finally, although the hygiene hypothesis is generally explained as a protective effect of early exposures resulting in long-lasting health benefits, some studies suggest that exposures throughout life may be important (and that long term continual exposure may be required to maintain optimal protection) (Douwes et al., 2007 and Douwes et al., 2004).
Hypoxia-inducible factor -1 and Allergic Inflammation.

Evidence points to the hypoxia-inducible factor (HIF) family of transcription factors as key modulators of innate immune function and allergic asthma. The HIF family of transcription factors enables cells to sense and respond to changes in oxygen tension which is a crucial function of all nucleated cells. Under hypoxic conditions, gene expression is either switched on or off by HIF family members (Nizet and Johnson 2009, Huerta-Yepez et al., 2011).

Dermal: Lung Sensitization

Atopic conditions such as atopic dermatitis and allergic asthma often afflict the same individual. Thus, it seems likely that these conditions may share common mechanisms. Indeed, a number of studies provide compelling evidence that skin barrier defects increase susceptibility to asthma and influence its severity. Skin barrier defects facilitate allergen absorption, which leads to systemic Th2-like sensitization and asthma (Dietert and Luebke, 2012).
Filaggrin is a protein involved in the terminal differentiation of the epidermis and the formation of the skin barrier (Candi et al., 2005). Genetic mutations of filaggrin (R501X and 2282del4) impair its ability to support epithelial barrier formation. Importantly, these two loss-of-function mutations were also associated with asthma in patients with co-existing atopic dermatitis. Furthermore, filaggrin mutations were significantly associated with increased asthma severity independent of concomitant eczema (Dietert and Luebke, 2012).

**Intestinal microbiota**

Ingestion of lactobacilli associated with raw milk consumption may be important as they can colonize the human gut and may be involved in immunomodulation during development (Bjorksten et al., 1999); however, various microbes of the gut flora or alterations in total gut microbiota may play a role as well (Bjorksten et al., 2001, Brooks et al., 2013).

Reduced intestinal biodiversity during infancy has been associated with increased allergy at school age (Abrahamsson et al., 2012). Similar findings of low-gut microbial diversity in allergic individuals have also been reported (Sjogren et al., 2009), and altering intestinal flora with antibiotics in murine models increases susceptibility to allergic airway inflammation (Russell et al., 2012). Furthermore, it has been suggested that the composition of maternal intestinal flora may be related to wheeze in infants, possibly through indirect exposure, or maternal transfer (Lange et al., 2012).

**Asthma-Susceptibility Genes**

Genetic association studies have identified and replicated susceptibility genes for asthma in 3 major types of studies: Candidate gene studies, Positional cloning using linkage studies and Genome-wide association studies (GWAS) (Scott, 2015).

**Clinical assessment and Investigations:**

1. **Criteria for making diagnosis of asthma**
   

   A history of variable respiratory symptoms:

   1. Typical symptoms are wheeze, shortness of breath, chest tightness, and cough.
   2. People with asthma generally have more than one of these symptoms
3. The symptoms occur variably over time and vary in intensity.
4. The symptoms often occur or are worse at night or on walking.
5. Symptoms are often triggered by exercise, laugh, allergens or cold air.
6. Symptoms often occur with or worsen with viral infections.

Evidence of variable expiratory airflow limitation:

1. At least once during the diagnostic process when FEV1 is low, document that the FEV1 / FVC ratio is reduced (normally more than 0.75-0.80 and more than 0.90 in children)

2. Document that variation in lung function is greater than in healthy people, for example:

   FEV1 increases by more than 12% and 200 ml (in children, ≥12% of the predicted value) after inhaling a bronchodilator, this is called bronchodilator reversibility.

   Average daily diurnal PEF variability is ≥ 10 % (in children, ≥ 13%)

   FEV1 increase by more than 12 % and 200 ml from baseline (in children by ≥ 12% of the predicted value) after 4 weeks of anti-inflammatory treatment (outside respiratory infection)

3. The greater the variation of pulmonary functions, or the more times excess variation is seen, the more likely the diagnosis is to be asthma

4. Testing may need to be repeated during symptoms, in the early morning or after withhold in bronchodilator medications

5. Bronchodilator reversibility may be absent during severe exacerbation or viral infections: if bronchodilator reversibility is not present when it is first tested, the next step depends on the clinical urgency and availability of other tests (2017 GINA report, Global Strategy for Asthma Management and Prevention).

   ii. How to assess asthma control

   According to 2017 GINA report, Global Strategy for Asthma Management and Prevention. Asthma control means the extent to which the effects of asthma can be seen in the patient, or have been reduced or removed by treatment. Asthma control has two domains symptoms control and risk factors for future poor outcomes.
Poor symptom control is a burden to patients and a risk factor for flare ups. Risk factors are factors that increase the patient future risk of having exacerbations, loss of lung function or medication side effects.

In the past 4 weeks, has the patient had: Daytime symptoms more than twice/week?; Any night waking due to asthma?; Reliever needed more than twice /week?; And any activity limitation due to asthma?. Well controlled when none of these is found, partially controlled when 1-2 of these were found and uncontrolled asthma when 3-4 of these are found.

iii. **Pulmonary function tests and bronchial provocation tests:**

One of the hallmarks of asthma is reversible airflow obstruction. A significant bronchodilator response is commonly seen in children with asthma after administration of albuterol, with FEV1 improving by at least 200 mL and 12%. The same criteria have been applied to adults; however, many older patients have reduced expiratory flow rates due to aging. Lung volumes can also improve but often are not measured after bronchodilator challenge (*Amir et al., 2012*).

Aging causes an annual decline in lung function and this may confound the differentiation between asthma and COPD. The normal decline in FEV1 is approximately 25 to 35 mL per year. From age 25 to 39, the decline is approximately 20 mL per year; age >45 years, 35 mL per year. Therefore, loss of spirometric lung function increases with age, emphasizing the need to consider the individual’s current age when evaluating lung function and not just the age of asthma onset (*Amir et al., 2012*).

Bronchoprovocation challenge tests detect the presence of non-specific airway hyperresponsiveness (AHR) in patients with a normal spirometry and chest X-ray. Airway hyperresponsiveness is defined as the degree to which expiratory flow rates decline after response to a nonspecific trigger (e.g., methacholine, histamine, cold air, Mannitol, exercise, or adenosine-5’-monophosphate). The methacholine (provocation concentration) PC20 is the provocative challenge or dose of methacholine that causes a 20% decrease in a patient’s baseline FEV1 measured after inhaling normal saline. Methacholine bronchial challenge is used primarily to rule out AHR in the clinical setting of normal pulmonary function tests (*Amir et al., 2012*).

iv. **Exhaled nitric oxide**

The measurement of nitric oxide in a patient's exhaled breath is being evaluated as a method that might aid in the diagnosis of asthma (**figure 5**). As part of bronchial inflammation, persons with asthma have upregulation of nitric oxide synthase in their
respiratory mucosal epithelium and generate increased amounts of nitric oxide in their 
exhaled breath. Low levels of nitric oxide are present in normal individuals (Massaro et 
al, 1995).

Some preliminary results have suggested sensitivity and specificity similar to 
methacholine bronchoprovocative testing; although one community-based study failed 
to confirm this degree of accuracy. The simplicity of the testing procedure and its 
usefulness even in the absence of airflow obstruction make the concept highly appealing 
(Smith et al., 1992).

The use of exhaled nitric oxide measurements (FENO) in clinical practice is 
coming of age. There are a number of theoretical and practical factors which 
have brought this about. Firstly, FENO is a good surrogate marker for 
eosinophilic airway inflammation. High FENO levels may be used to 
distinguish eosinophilic from non-eosinophilic pathologies. This information 
complements conventional pulmonary function testing in the assessment of 
patients with non-specific respiratory symptoms. Secondly, eosinophilic airway 
inflammation is steroid responsive. There are sufficient data to justify the claim 
that FENO measurements may be used successfully to identify and monitor 
steroid response as well as steroid requirements in the diagnosis and 
management of airways disease. FENO measurements are also helpful in 
identifying patients who do or do not require ongoing treatment with inhaled 
steroids. Thirdly, portable nitric oxide analysers are now available, making 
routine testing a practical possibility. However, a number of issues still need to 
be resolved, including the diagnostic role of FENO in preschool children and the 
use of reference values versus individual FENO profiles in managing patients 
with difficult or severe asthma (Taylor et al., 2007).
Figure 5: (a) Healthy airways produce a relatively low level of NO under the control of chemical messengers not affected by the use of ICSs. (b) In the asthmatic airway, inflammatory mediators, which can be downregulated by ICSs, are released in response to asthma triggers. These mediators increase the level of inducible NO synthase (iNOS), which in turn, increases the production of NO.

v. Biomarker Technologies

The collection and analysis of exhaled breath condensate (EBC) is a recent development in the world of asthma. The EBC contains water vapor, respiratory droplets, particles, and likely thousands of molecules released from the lung (Gershwin and Albertson, 2012)
Many biomarkers of airway inflammation have been reported in the EBC. Amongst the compounds measured markers of oxidative stress (8-isoprostane and hydrogen peroxide), leukotrienes (B4 and cysteinyl), nitrosothiols, chemokines (e.g., eotaxin-1), cytokines (e.g., IL-6) and isoprenes. Although at present it is unclear whether EBC will have the ability to distinguish between asthma and COPD, or to help diagnose asthma, it is an active area of research with significant promise (Gershwin and Albertson, 2012)

The role of indoleamine 2,3-dioxygenase (IDO), and enzyme that mediates the catabolism of tryptophan, remains a controversy in its role to detect allergic entity of bronchial asthma and its correlation with asthma control level. Atopic patients with bronchial asthma have high serum IDO levels. On the other hand, serum IDO level did not differ significantly between uncontrolled and controlled asthma patients so there is no relationship between IDO levels and asthma control (Refaat et al., 2016).

vi. Tests for allergy

If a patient reports that exposure to typical allergens causes asthmatic symptoms, then positive allergy tests will have a greater positive predictive value. Aeroallergens are the types of allergens most commonly implicated in asthma. Food allergens rarely cause isolated asthmatic symptoms. By asking relatively few specific questions, clinicians can often elicit the relationship between allergen exposure and asthmatic symptoms (Massaro et al, 1995). Allergic sensitivity to specific allergens in the environment can be assessed using two methods: allergy skin tests and blood tests for allergen-specific IgE (Massaro et al, 1995).

In Prasad et al. study, skin sensitivity to various allergens in patients of nasobronchial allergy were studied and 2880 skin prick tests with 60 allergens were performed in 48 patients of nasobronchial allergy; their results reveal that most common offending allergens were insects (21.2%), followed by dusts (12.0%), pollens (7.8%), animal dander (3.1%), and fungi (1.3%). Common dust allergens were house dust, wheat dust, cotton mill and paper dust. Among pollens, Amaranthus spinosus, Argemone mexicana, Adhatoda vasica, Ailanthus and Cannabis were found to be common allergens. Among fungi, Aspergillus fumigatus, Aspergillus flavus, Alternaria teneis and Fusarium sodani were common allergens. Their data may prove useful in allergen avoidance and immunotherapy in these patients (Prasad et al., 2009).

From 2nd WAO International Scientific Conference (WISC 2012) in India at December 2012, Giriyanna et al. study was presented, Skin Prick Test was done in 139 patients suffering from bronchial asthma diagnosed based on GINA guidelines. Skin Prick Test was performed using 49 allergens extracts. Allergen extracts included 19 pollens, 10 fungi, 5 dusts, 2 dust mites (Dermatophagoides farinae and Dermatophagoides pteronyssinus), 10 insects and 3 epithelia (Giriyanna et al., 2012). Results out of 139 patients who underwent skin prick test, 40% (56) were males and
60% (83) were females. Majority (60%) were in the age group of 21 - 40 years. 43% (60) had family history of asthma/atopy, 80% (111) had allergic rhinitis, 24% (34) had chronic urticaria and 24% (33) had allergic conjunctivitis. Out of 139 patients, 100 (71.94%) were sensitive for one or more allergens. The common offending allergens found in the study were dust mites (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*) - 49.28%, dusts - 7.2%, pollens - 6.77%, insects - 6.62%, fungi - 4.53%, epithelia - 1.92% (*Giriyanna et al., 2012*).

**vii. Blood tests**

No blood tests are available that assess the presence or absence of asthma or gauge its severity. However, a complete blood count (CBC) with differential white blood cell analysis to screen for eosinophilia or significant anemia may be helpful in certain cases as markedly elevated eosinophil percentages (>15%) may be due to allergic asthma, but should prompt consideration of alternative diagnoses, including parasitic infections (eg, Strongyloides), drug reactions, and syndromes of pulmonary infiltrates with eosinophilia (*Massaro et al., 1995*).

**Treatment**

The long-term goals of asthma management are to achieve good symptom control, and to minimize future risk of exacerbations, fixed airflow limitation and side-effects of treatment. The patient’s own goals regarding their asthma and its treatment should also be identified. Effective asthma management requires a partnership between the person with asthma (or the parent/carer) and their health care providers. A study by Bruzzese et al assessed the Asthma Self-Management for Adolescents (ASMA) approach, which is a school-based intervention for adolescents and medical providers. The study found that ASMA helped improve self-management and reduced morbidity and urgent health care use in low-income, urban, minority adolescents (*Bruzzese et al., 2011*).

According to all versions of GINA Report, Global Strategy for Asthma Management and Prevention, once asthma treatment has been commenced, ongoing treatment decisions are based on a cycle of assessment, adjustment of treatment, and review of the response. Controller medication is stepped up or down in a stepwise approach to achieve good symptom control and minimize future risk of exacerbations, fixed airflow limitation and medication side-effects. Once good asthma control has been maintained for 2–3 months, treatment may be stepped down in order to find the patient’s minimum effective treatment.
Inhaled Corticosteroids (ICS) remain the single most effective therapy for adult patients with asthma. It is the first line treatment for asthmatics; however, it is increasingly evident that predicting ICS response is difficult where neither sputum eosinophils nor FENO are predictive of improvement. Only lung function, specifically FEV1 and FEV1/FVC were predictive of a favorable response (NIH-NAEPP-EPR3, 2007).

Medical care includes treatment of acute asthmatic episodes and control of chronic symptoms, including nocturnal and exercise-induced asthmatic symptoms. Relief medications include short-acting bronchodilators, systemic corticosteroids, and ipratropium. The management includes the use of control agents such as inhaled corticosteroids, inhaled cromolyn or nedocromil, long-acting bronchodilators, theophylline, leukotriene modifiers, and more recent strategies such as the use of anti-immunoglobulin E (IgE) antibodies (omalizumab) and mepolizumab (anti-interleukin-5 treatment). One such target is IL-5, a type-1 cytokine that is central to the initiation and sustenance of eosinophilic airway inflammation (Mukherjee, 2014).
**Allergic Rhinitis**

**Definition and classification:**

Rhinitis is characterized by 1 or more of the following symptoms: nasal congestion, rhinorrhea (anterior and posterior), sneezing, and itching. Rhinitis is usually associated with inflammation, but some forms of rhinitis such as vasomotor rhinitis or atrophic rhinitis are not predominantly inflammatory. Rhinitis frequently is accompanied by symptoms involving the eyes, ears, and throat (Wallace et al., 2008).

Some investigators prefer the term rhinosinusitis to the separate terms rhinitis and sinusitis. This is because rhinitis and sinusitis frequently occur together; rhinitis commonly leads to sinusitis; nasal symptoms are common with sinusitis; and the nose and sinus mucosa are contiguous (Hamilos, 2010).

Rhinitis is classified as allergic or nonallergic, but not all types of rhinitis can be easily separated into one of these categories. For example, occupational rhinitis has been classified separately from allergic and nonallergic because it may have components of both allergic and nonallergic rhinitis. Conditions that mimic symptoms of rhinitis include nasal polyps, cerebrospinal fluid rhinorrhea, ciliary dyskinesia syndrome, and structural/mechanical factors, such as deviated septum and pharyngonasal reflux (Wallace et al., 2008).

There is no generally accepted method of grading rhinitis severity. The severity of allergic rhinitis has been classified as “mild” or “moderate/severe” depending on the severity of symptoms and quality-of-life outcomes. An international working group (Allergic Rhinitis and its Impact on Asthma [ARIA]) has proposed a classification for allergic rhinitis that placed patients into 1 of 4 categories: (1) mild intermittent, (2) mild persistent, (3) moderate/severe intermittent and (4) moderate/severe persistent (Bousquet et al., 2006).

This classification system discarded the terms seasonal and perennial, emphasizing that an aeroallergen (e.g. grass pollen) that occurs seasonally in one region may be detected throughout the year in another geographical area. The ARIA definition of mild rhinitis may be a useful comparative reference point for other severity grading schemes; this states that none of the following items is present: sleep disturbance; impairment of daily activities, leisure, and/or sport; impairment of school or work; and symptoms present but not troublesome (Bousquet et al., 2001).
Risk factors:

The following are proposed or identified risk factors for allergic rhinitis (Matheson et al., 2011):

1. Family history of atopy (ie, the genetic predisposition to develop allergic diseases)
2. Male sex
3. Birth during the pollen season
4. Firstborn status
5. Early introduction of formula and food
6. Early use of antibiotics
7. Maternal smoking exposure in the first year of life
8. Exposure to indoor allergens, such as dust mite allergen
9. Serum IgE >100 IU/mL before age six
10. Presence of allergen specific IgE

Pathophysiology of allergic rhinitis

Sensitization to allergens

Antigen presenting cells (APCs), such as dendritic cells in the mucosal surface, process allergens and present some peptides from allergens on the major histocompatibility complex (MHC) class II molecule (Chaplin et al., 2006). This MHC class II molecule and antigen complex take a role as the ligand of T-cell receptors on Naïve CD4+ T cells, which result in differentiation of Naive CD4+ T cells to allergen-specific Th2 cell. Activated Th2 cells secret several cytokines, which induce isotype switching of B cells to produce specific IgE and proliferation of eosinophils, mast cells and neutrophils. Produced antigen-specific IgE binds to high-affinity IgE receptors on mast cells or basophils (Broide et al., 2007).

Early and late reactions

When AR patients are exposed to allergens, allergic reactions develop in 2 different patterns according to time sequence. One is the early reaction, in which sneezing and rhinorrhea develops in 30 minutes and disappears. The other is the late reaction, which shows nasal obstruction approximately 6 hours after exposure to allergens and subsides slowly. The early reaction is the response of mast cells to offending allergens (type I hypersensitivity) (Yang-Gi Min, 2010).
Stimulated mast cells induce nasal symptoms by secreting chemical mediators such as histamine, prostaglandins and leukotrienes (Prussin and Metcalfe et al., 2008). In contrast to the early reaction, eosinophil chemotaxis is the main mechanism in the late reaction, which is caused by chemical mediators produced in the early reaction. Several inflammatory cells, eosinophils, mast cells and T cells migrate to nasal mucosa, break up and remodel normal nasal tissue, and these processes result in nasal obstruction which is the main symptom of AR patients (Kay et al., 2001).

**Neurogenic inflammation**

When respiratory epithelium is destroyed and nerve endings are exposed by cytotoxic proteins from eosinophils, sensory nerve fibers are excited by nonspecific stimuli and stimulate both sensory afferent and surrounding efferent fibers, the so called retrograde axonal reflex. This makes the sensory nerve fibers secrete neuropeptides such as substance P and neurokinin A, which induce contraction of smooth muscles, mucous secretion of goblet cells and plasma exudation from capillaries. This process is called neurogenic inflammation (Togias, 2000).

**Non-specific hyperresponsiveness**

Non-specific hyperresponsiveness is one of the clinical characteristics of allergic inflammation. Due to eosinophilic infiltration and destruction of nasal mucosa, the mucosa becomes hyperactive to normal stimuli and causes nasal symptoms such as sneezing, rhinorrhea, nasal itching and obstruction (Yang-Gi Min, 2010). This is a non-immune reaction that is not related to IgE. Hypersensitivity to non-specific stimuli such as tobacco or cold and dry air as well as specific allergens increases in AR patients (Gerth van Wijk RG, 1999).

Also nasal *staphylococcus aureus* actively modulated the immune reaction in persistent allergic rhinitis patients by promoting local IgE production, and they recommend early detection and treatment of *staphylococcus aureus* carriage in patients (Refaat et al., 2005).

**Diagnosis of allergic rhinitis**

The diagnosis of AR is based on a typical history of allergic symptoms and diagnostic tests. When 2 or more symptoms out of watery rhinorrhea, sneezing, nasal obstruction and nasal pruritus persist for ≥1 hour on most days, AR is strongly suspected and a confirmative diagnosis should be established by the skin prick test or the serum-specific IgE level (Bousquet, 2008).
Unilateral nasal stuffiness, mucopurulent rhinorrhea, mucoid postnasal drip, pain, recurrent epistaxis or anosmia is usually not associated with AR (Yang-Gi Min, 2010).

Local allergic rhinitis (LAR) is a newly described type of rhinitis, a common among rhinitis patients in Egypt but still misdiagnosed. It can affect patients previously diagnosed as non-allergic rhinitis. Refaat et al., found that eighty percentages of the rhinitis patients that enrolled in their study showed positive skin prick test and high serum total IgE, and these were considered allergic rhinitis patients (AR) with systemic atopy. For the remaining 20%; positive nasal allergen provocation (NAPT) test was obtained in 62.5% of them, which represent local allergic rhinitis (LAR) patients while 37.5% were negative to NAPT which represent non allergic rhinitis (NAR) patients (Refaat et al., 2015).

Skin testing

Skin testing is the most important to find offending allergens (figure 6). There are various testing methods including the scratch, prick/ puncture, intradermal and patch tests. Among them, the skin prick test is usually recommended in clinical practice. False positive or false-negative reactions are frequently evoked in skin tests, which means that positive reactions to specific allergens in skin tests does not always have a direct correlation with actual allergic reactions in the nasal cavity. There is controversy regarding the interpretation of the test results, and criteria for positivity are different among allergy clinics (Yang-Gi Min, 2010).

![Figure 6: Skin prick test and intradermal test](Adopted from Korean Academy of Pediatric Allergy and Respiratory Disease).
Serum specific IgE level

Although the radioallergosorbent test (RAST) was the first method to detect serum-specific IgE, this test has not been widely used because it requires a radioactive isotope and expensive equipment and also because this test cannot detect multiple antibodies simultaneously (Yang-Gi Min, 2010).

The next method is the multiple allergen simultaneous tests (MAST). Since the MAST has some advantages over the RAST, it has been widely used. The MAST uses a photo reagent instead of a radioactive isotope, does not require expensive equipment and can detect multiple allergens simultaneously. This test is not affected by drugs such as antihistamines, is less invasive and can be adopted in patients with dermographism. One problem with the MAST is a low sensitivity as compared to the skin prick test (Yang-Gi Min, 2010).

Overview of management:

As more medications become available without a prescription, patients can extensively self-treat, although the side effects of some over-the-counter allergy medications can be significant. Therefore, the current challenge for clinicians is to assure that both patients with moderate to severe allergic rhinitis are adequately treated with medications that do not cause undue side effects. Management of allergic rhinitis involves the following components:

- Allergen avoidance
- Pharmacotherapy
- Allergen immunotherapy (when appropriate)

Available medications

Most patients require pharmacotherapy, in addition to allergen avoidance, for satisfactory symptom control. The most effective single therapy for patients with persistent and significant nasal symptoms is a glucocorticoid nasal spray. Other therapies include oral antihistamines, antihistamine nasal sprays, mast cell stabilizers (the cromoglycates), leukotriene modifiers, and ipratropium. In contrast, nasal decongestant sprays and systemic glucocorticoids should not be used for routine treatment of allergic rhinitis (Platts-Mills, 2011).

Allergen avoidance is appropriate for symptomatic patients with allergic responses documented either with positive skin tests or serum assays for specific IgE antibodies. Once specific sensitivities have been defined, it is important to implement a comprehensive environmental control plan for all, or as many as possible, of the
allergens that are relevant to that patient. Studies in which environmental control has been most effective are those in which the specific measures taken were tailored to the patient and addressed the major allergens to which each patient was sensitized (Morgan et al., 2004)

Patient/parent education is essential for successful environmental modification. Simply handing a sheet of recommendations to a patient, without providing detailed education about the proposed measures or specific follow-up, is unlikely to be effective. In addition, the clinician should be mindful that major environmental modifications, such as removing carpeting, modifying heating systems, or replacing old upholstered furniture, are expensive and may not be affordable for some families. Recognition of this and an initial emphasis on low-cost interventions should enhance patient cooperation (Platts-Mills, 2011).

**Immunotherapy**

Immunotherapy is the only therapeutic option that modifies the basic allergic mechanism by inducing desensitization and producing an anergy state for offending allergens. Immunotherapy was initially introduced for seasonal AR due to pollens. At present, its indications have been extended to other allergic diseases due to hymenoptera, house dust mite, animal dander or fungi (Cohen and Evans, 2004).

Extracts of offending allergens are injected subcutaneously with increasing doses until a maintenance dose is reached. The maintenance dose is administered for ≥3 years. Although subcutaneous immunotherapy is a well-established treatment option, the risk of anaphylaxis has led to the development of other administration routes such as the oral, sublingual or nasal route. Sublingual immunotherapy (SLIT) has been used for 20 years in European countries because of its non-invasiveness, low incidence of adverse effects and convenience of self administration. Even it has replaced subcutaneous immunotherapy in some countries (Platts-Mills, 2011).
CHAPTER FOUR

Aeroallergen and respiratory allergic diseases
Aeroallergen and respiratory allergic diseases

Introduction

Aeroallergens are relatively large and complex particles, such as pollens, moulds, insect parts, animal dander, plant fragments, and house dust mites that are capable of eliciting allergic reactions in susceptible persons. These particles contain many molecular components, only some of which are antigenic (Neaville et al., 2002).

When specific antigenic components have been identified, they usually are proteins with some carbohydrate subunits and have a molecular weight of 10,000 to 40,000 D. The antigenicity of these molecules is fundamentally a property of their size, spatial configuration, and chemical groupings. The overall allergic importance of these particles is not only a function of their antigenicity, but also of their availability in the environment for contact with susceptible persons and the suitability of particle size for impingement on the respiratory mucosa (Neaville et al., 2002).

Immediate hypersensitivity to allergens is very common among children and young adults with asthma and rhinitis. Sensitization to one or more of the major indoor allergens (such as dust mite, cat, dog, or cockroach), combined with significant accumulation of relevant allergens in the house, has been consistently found to be the strongest risk factor for asthma in population, case control, and prospective studies (Platts-Mills et al., 1997).

Further evidence for a causal relationship between allergen exposure and asthma comes from bronchoprovocation experiments demonstrating that these allergens can induce bronchospasm, eosinophilic airway inflammation, and prolonged increases in bronchial hyperreactivity (Cockcroft et al., 1979). Perhaps more significantly, moving some asthmatic children or adults from their homes to a different, low allergen residential setting results in major improvements in clinical symptoms and bronchial hyperreactivity (Piacentini et al., 1996). This background provides a powerful rationale for recommending that allergic patients should reduce allergen exposure in their houses as part of the management of asthma and allergic rhinitis (Sharma et al., 2007).

Specific monoclonal antibody-based assays have been developed to monitor allergen levels during controlled trials and to test the specific measures recommended to control exposure to mite, cat, dog, and cockroach antigens (Erwin et al., 2005). These techniques have facilitated detailed studies of specific allergens and have helped to define effective control measures, although the exquisite specificity of these measurements may mean that some forms of the specific allergen (e.g., Dust mites; Der p 1) may escape detection (Van Ree, 2007).
A number of small controlled trials have successfully documented a decrease in mite allergen for six months or more following a range of interventions (Woodcock et al., 2003, Gotzsche et al., 1998). Each of these studies reported benefit, and four studies found a highly significant decrease in nonspecific bronchial hyperreactivity. It is important to note that over half of the reported trials of dust mite avoidance have failed because the measures proposed did not reduce allergen exposure for a significant period of time (Gotzsche et al., 1998). There are several conclusions from these studies (Platts-Mills et al., 2013):

i. Successful controlled trials have used combinations of physical measures, including pillow covers, mattress covers, washing bedding in hot water, and carpet removal, rather than chemical treatments.

ii. At least three to six months of sustained intervention was necessary to demonstrate clinical benefit. Thus, patients should be encouraged to adopt dust mite control measures that they can effectively sustain over time, and they should be advised that symptoms are expected to improve gradually.

**Indoor and outdoor aeroallergens**

Indoor moulds and fungi are most problematic in homes with high humidity, standing water, or water damage. If the home of a mould-allergic patient contains visible mould or smells of mould, then remediation for this allergen is in order. Outdoor allergens, especially pollens and moulds, are difficult to avoid short of limiting contact with the outside world. Patients with pollen and mould allergies should be advised to close the windows at home and also in the car, stay indoors when possible, and use air conditioners to filter the air during times of peak symptoms. Showering before bed to remove allergens from hair and skin can help reduce contamination of the bedding. Over the counter saline sprays and rinses can be used to wash allergens from the nasal lining after outdoor exposure (Platts-Mills et al., 1997).

Even sometimes these aeroallergens could be considered as a possible etiology of chronic urticaria, even if not associated with allergic respiratory diseases. Among patients with chronic idiopathic urticaria, the most common aeroallergens were mites (13.8%), followed by pollens (5.2%), and mixed moulds (4%) in the study conducted by Refaat et al. (Refaat, 2010)
Pollen grains

Pollen grains are male reproductive structures of seed-bearing plants and function to carry the male gametes (sperm) to the female gametes (egg), which remain on the plant. Pollen transfer for plants with showy, colorful, and fragrant flowers is accomplished by insects (entomophily). In these instances, the pollen is often large, with an adhesive coating. Remarkable adaptations of some plants allow dissemination of pollen by birds, bats, mice, or even snails (Neaville et al., 2002).

Most pollens of allergic importance are windborne (anemophily). Plants with windborne pollen transfers are typically drab, with small, inconspicuous, and odorless flowers. Their pollen is usually small and nonadhesive, with a smooth unsculptured surface. Most pollen is shed in the early morning hours, but dispersal by wind currents usually produces maximal pollen concentrations in the afternoon or early evening. Although pollen grains are viable for only a few hours, nonviable pollen is still an active allergen. A gentle wind can carry pollen of anemophilous plants for many miles and produce high pollen concentrations in urban and metropolitan areas, far from their rural or suburban source (Neaville et al., 2002).

Dust Mites

Dust mites (Dermatophagoides pteronyssinus and Dermatophagoides farinae) are arachnids that colonize bedding, sofas, carpets, or any woven material. Dust mites do not bite, and aside from causing allergic disease, are not known to pose other harm to humans. It is sometimes difficult to educate patients regarding dust mites because neither the mites, nor their debris, can be seen under normal circumstances (Platts-Mills et al., 1997).

Mites absorb humidity from the atmosphere and feed on organic matter (including shed human and animal skin particles), usually with the aid of fungal degradation. They require nests to live in, a source of food (rarely a limiting factor when these organisms live indoors), and sufficient humidity. Dust mite infestation is far less common in arid and high-altitude climates, such as the mountain states and southwestern United States (Platts-Mills et al., 1997).

Refaat M.et al. studied the role of house dust mites (HDM) in allergic and non allergic nasal diseases and found that in addition to its role in atopic rhinitis with release allergens, HDM also may share in the pathogenesis of non AR since most of HDM proteolytic enzymes were detected in non AR patients as similar to AR patients due to HDM (Refaat et al., 2014).
Airborne fungi

Airborne fungi are significant environmental components involved in the etiology of allergic respiratory diseases (Simon-Nobbe et al., 2008). Aspergillus, Cladosporium, Alternaria, Curvularia and Fusarium species have been reported as the most prevalent components of indoor and outdoor aeromycota around the world (Fang et al., 2005). The incidence of fungal sensitization in patients of allergic respiratory diseases has been reported from 2.3% to even 80% in various studies worldwide (D’Amato et al., 1997).

A randomized trial of indoor mould remediation in patients with asthma and visible mould growth in the home demonstrated benefit, regardless of whether patients were sensitized to four common allergenic moulds by skin prick testing or not. In this study, 164 homes housing 232 asthmatic patients were randomly assigned to undergo cleaning with detergent and fungicide and installation of an attic fan, or to have this intervention performed one year later. There was a dramatic difference between the two groups at six months, with improvement in breathing symptoms in 52% and 0% of patients in the intervention and control groups, respectively (Burr et al., 2007).

Indoor Pets

Indoor pets are a common source of allergens, and the vast majority of pet-allergic patients are reactive to cats, dogs, or both. However, a growing number of exotic animals are kept as pets, including reptiles, birds, insects, rodents, ferrets, and monkeys, and allergic responses to these animals are also reported (Phillips and Lockey, 2009).

The most effective measure in controlling allergens derived from animals is to persuade the family or patient not to keep animals in the house. Scales shed from the animal's skin are the major source of animal allergens. Keeping a pet outdoors is effective, but restricting the animal to one part of the house is ineffective because animal allergens, particularly those from cats, are easily carried on clothing. Both cat and dog allergens can remain airborne for extended periods of time due to carriage on particles that, because of their small size, settle very slowly. Because so many patients refuse to give away their animals, efforts have been made to control cat allergen while the animal remains in the house. The clinical effectiveness of these measures is not well established, and patients must understand that the presence of a cat in the house represents such a large source of allergen that none of the proposed measures can consistently control allergen exposure (De Blay et al., 1991).

Cat allergen is transferred on clothing and can easily be detected in schools and in houses without a cat (Gelber et al., 1993). The quantities found in these sites are sometimes surprisingly high, such as 80 mcg per gram of dust of the antigen Fel d 1 (the major cat allergen) or Can f 1 (the major dog allergen). In some cases, this is well
within the range of allergen concentration found in homes with an animal. Furthermore, this passively transferred allergen can become airborne and cause symptoms (Almqvist et al., 2001).

Fungal Allergens

Fungi are eukaryotic, unicellular or multicellular, plantlike organisms that do not contain chlorophyll. They are mostly spore-bearing organisms that exist as saprophytes or parasites of animals and plants. Moulds have been defined as a furry growth of microscopic fungi or fungi that produce microscopic reproductive structures. Thus, fungi and moulds are not entirely synonymous, but the terms are often used interchangeably (Volcheck, 2009).

The role of fungi in producing allergic and respiratory symptoms is well established and has been described since the early 1700s. More recently, fungal sensitivity has been shown with skin-test reactivity and inhalation challenge studies. Spore concentrations of Alternaria equal or greater than 100 spores/m³ are believed to evoke allergic symptoms, whereas the reference value for Cladosporium is estimated to be 3,000 spores/m³. Although fungal spores are thought to be causative agents of allergic reactions, other particles that become airborne, including mycelia fragments may trigger an allergic reaction. Fungal spores are structurally different from pollens because the inhaled particles consist of entire living cells capable of growing and secreting allergens in vivo (Volcheck, 2009).

Classification of fungi

It has been estimated that around 1 to 1.5 million fungal species exist worldwide, but so far only 80,000 have been described (Mari et al., 2003). Among these, about 80 mould genera have been shown to induce type I allergies in atopic individuals (Simon-Nobbe, 2008).

The major taxonomic groups of fungi that are currently recognized are the ascomycetes, basidiomycetes, and zygomycetes. Some texts also refer to a separate group, the deuteromycetes, or Fungi Imperfecti. The deuteromycetes are an artificial grouping of asexual fungal stages and are primarily forms of ascomycetes that produce asexual spores called conidia. Deuteromycetes (Fungi Imperfecti) contains a large number of allergenic fungi that reproduce asexually by the differentiation of specialized hyphae called conidiophores. The species are characterized by the morphology of the conidia. The asexual ascomycetes (Deuteromycetes) include Aspergillus, Penicillium, Alternaria, Cladosporium, Fusarium, Epicoccum, Drechslera, and Curvularia (Volcheck, 2009).
Perhaps more useful to allergy is the ecological groupings of fungi. These groupings pair fungi that sporulate in response to similar environmental conditions. This results in high spore counts of these types, with a similar exposure. The three general ecological types of fungi are the phyllophane fungi, basidiomycetes, and the soil and litter fungi. Phyllophane fungi live on the surface of leaves, and most of them are deuteromycetes. Known allergens in this group are *Cladosporium*, *Alternaria*, *Epicoccum*, and *Curvularia*. Because phyllophane fungi live on the leaf surface, they have adapted to continual wetting and drying cycles, sunlight exposure, and exudates from the leaves. This hardiness also allows for survival in the indoor environment. Patients with a strong allergy to *Alternaria*, for example, may want to avoid walking in or near the woods on these days. Peak levels are typically found in the early afternoon, after the organisms have dried from the morning dew (Volcheck, 2009).

Basidiomycetes include mushrooms, puffballs, and conks. Several basidiomycetes are known to produce allergens. Basidiomycetes typically live on plants or as decomposers of wood and are found primarily in shade trees, lawns, parks, or wherever wood becomes sufficiently wet to decay. Basidiomycetes also include rust and smut fungi. The prevalence of reactivity to basidiomycetes appears comparable to that of deuteromycetes, but very few commercial extracts are available (Horner et al., 1995).

The soil and litter fungi are primarily deuteromycetes. Well-known members of this group are *Penicillium*, *Aspergillus*, and *Fusarium*. *Penicillium* and *Aspergillus* are considered the most common indoor allergens, but they also are measured outdoors. These fungi can be found in moist soil, wet leaves, or other decaying organic matter. They are found indoors in indoor dust, particularly in high humidity. When cellulose building materials, for example, wallpaper, paper coating on wallboard, and acoustic ceiling tiles, are wet, they provide an environment for the fungi to flourish. Most soil and litter fungi passively release their spores. Disturbing mulch or decaying organic matter releases plumes of spores from soil and litter fungi. Patients allergic to *Aspergillus* or *Penicillium* should avoid old wet piles of leaves and avoid handling wet yard wastes and composting activities. The rest of this section focuses on the characteristics of commonly encountered (Volcheck, 2009).

**Allergenic fungi and exposure to fungal allergens**

Indoor and outdoor exposure to fungal components, including spores, is a recognized triggering factor for respiratory allergy and asthma as well as for atopic dermatitis (Green, 2006).

The most important allergenic fungi belong to the genera *Alternaria*, *Aspergillus*, and *Cladosporium* (Crameri, 2006), whereas members of the genera *Candida*,
Penicillium, Clavularia and others seem to be, with the exception of the genus Malassezia in patients suffering from atopic dermatitis, less important as allergenic sources (Mari, 2003). Also the exact prevalence of fungal sensitization among the general population is still unknown (Crameri et al., 2006).

The best estimates of fungal sensitization among allergic individuals come from a large skin prick test (SPT) survey on a cohort of subjects with respiratory diseases conducted with extracts from Alternaria, Aspergillus, Candida, Cladosporium, Penicillium, Saccharomyces and Trichophyton and indicate a prevalence of sensitization around 19% (Mari et al., 2003).

**Clinical manifestations of fungal hypersensitivity**

The clinical spectrum of hypersensitivity reactions elicited by fungi is very broad and includes, besides IgE-mediated type I allergy, reactions of types II, III and IV according to the old definition of Coombs and Gell (Coombs and Gell, 1963). Although the classification into types I to VI is still widely used in clinical practice, it should be mentioned that the reality is more complex because frequently several mechanisms operate together in the pathogenesis of hypersensitivity reactions, and this is especially true for reactions to fungi.

Fungal type I allergy is induced by a large number of fungal genera, the most important ones belonging to the ascomycota followed by basidiomycota and zygomycota (Simon-Nobbe et al., 2008). Clinically, the IgE-mediated sensitization to fungal allergens can manifest as allergic rhinitis and rhinosinusitis, allergic asthma and atopic dermatitis (Holgate et al., 2012).

The list of fungal allergens officially approved by the Nomenclature Subcommittee of the International Union of Immunological Societies (IUIS) spans 105 iso-allergens and variants from 25 fungal species belonging to the Ascomycota and Basidiomycota phyla. However, the number of fungal proteins able to elicit type I hypersensitivity reactions described in the literature is much longer, even if many of these allergens are poorly characterized (Agarwal and Gupta, 2011).

a. *Alternaria alternate*

*Alternaria alternata*, a member of the imperfect fungi, is one of the most important among all allergenic fungi and also an ascomycete and deuteromycete. The spores produced by imperfect fungi vary in shape, size, texture, color, number of cells, and thickness of the cell wall. *Alternaria* has dark “dictyospores,” with both horizontal and vertical septa. Airborne fungal spores of *Alternaria* and *Cladosporium* are found
throughout the world. *Alternaria* is found in the air year round, with peak levels from late summer to autumn. *Alternaria* thrives best on plants in the field and decaying plant parts in the soil. Satisfactory substrates are found in the soil, on foods, and on textiles. (Achatz et al., 1995).

The airborne spores are generally considered to be important causes of both allergic rhinitis and allergic asthma. There is an association between *Alternaria* sensitization and life-threatening asthma (Achatz et al., 1995).

The major allergenic fraction Alt a 1 has been cloned, but its biological function is unclear. Alt a 1, a major allergen of *Alternaria*, binds to the IgE of more than 80% of asthmatic patients with allergy to this fungus. About 90% of *Alternaria* -sensitive patients reacts positively to skin testing with this fraction. Minor allergens of *A. alternata* have also been isolated and characterized, including Alt a 2, Alt a 3, Alt a 4, Alt a 6, Alt a 7, Alt a 10, Alt a 11, and Alt a 12 (Vailes et al., 2001).

Response rates to these allergens are in the range of 10 to 60%. Cross reactivity among moulds is difficult to assess because it is unclear how much protein similarity that is present between life stages. Varying degrees of cross-reactivity have been shown between *Alternaria, Stemphylium*, and *Curvularia* (Volcheck, 2009).

Figure 7: *Alternaria alternata* showing branched acropetal chains and multicelled

b. *Aspergillus species*

*Aspergillus* species are saprophytic fungi widely distributed in nature and are associated with a number of human diseases (Kurup et al., 1991).
Asp f 1, a ribotoxin that inhibits protein translation, was found to be toxic to Epstein-Barr virus (EBV)–transformed (Moser et al., 1992). This allergen showed positive skin test reactivity in 80% of ABPA patients and 50% of asthmatic patients. However, because of the high toxicity and reactivity with skin test positive asthmatics and some normals, the usefulness of this allergen in diagnosis is questioned.

*Aspergillus fumigates* major allergen Asp f 2 is a 310 amino acid protein that has a fibrinogen-binding property (Banerjee et al., 1998). Asp f 2 reacted strongly with IgE antibody from ABPA patients. Approximately 87% of ABPA patients from the United States and Switzerland carried IgE antibody to Asp f 2 in their sera, whereas only very few skin test positive asthmatics and normal controls carried the IgE antibody. Also more than 80% of ABPA patients carried IgE to Asp f 3, whereas only a few asthmatics carried IgE (Kurup et al., 2000).

Asp f 4, an intracellular protein allergen with unknown biochemical function, showed strong reactivity with IgE from sera of cystic fibrosis (CF) patients with asthma or ABPA (Crameri et al., 1998). Asp f 5, a metalloproteinase, demonstrated skin test reactivity in more than 70% of allergic asthmatics without ABPA, whereas Asp f 5 showed reactivity in more than 90% of ABPA patients (Kurup et al., 2000). Although its diagnostic significance is not clear, Asp f 5 might be used as an indicator for sensitization to *Aspergillus*. Asp f 6, manganese superoxide dismutase (MNSOD), has a strong homology to MNSODs of human, bovine, fruit fly, gum tree, yeast, and E. coli. Asp f 6 reacted positively in skin tests and bound to IgE in the sera of ABPA patients. Recently its usefulness as a significant allergen for detecting IgE antibody in the sera of ABPA patients has been reported (Mayer et al., 1997).

Other minor allergens isolated from *A. fumigatus* and related *Aspergillus* spp. also bound to the IgE antibody from ABPA and allergic asthma patients. Several of these *Aspergillus fumigatus* allergens also exhibited high sequence homologies with known, functional proteins and enzymes (Shen et al., 1999).

Figure 8 : Conidiophores of Aspergillus. It may be *Aspergillus fumigatus.*
c. *Cladosporium herbarum*

*Cladosporium herbarum*, a deuteromycete, is widely distributed in the environment and is a major source of fungal inhalant allergens. Whereas *Alternaria alternata* is found more prominently in warm, humid climates, *Cladosporium* is the leading fungus in cooler climates, particularly Scandinavia. *(Viswanath, 2003).* *Cladosporium* is considered a “field fungus” and requires a substrate with relatively high moisture content. Approximately 60 antigens from *Cladosporium herbarum* have been identified by crossed immunoelectrophoresis, and approximately 36 of them have been shown to react with IgE from patients’ sera. It is prevalent from spring through the autumn, and levels decrease sharply with the first hard frost *(Akurst and Borch, 1979).*

Three major *Cladosporium herbarum* allergens have been purified and characterized: Cla h 1, Cla h 2, and Cla h 4. *Cladosporium* contains enolase, a highly conserved major allergen in most fungi. Approximately 25–50% of patients allergic to *Alternaria* and *Cladosporium* show IgE binding to enolase. Enolase has been found to be a highly conserved major allergen in most fungi, and might contribute to allergen cross-reactivity in mould allergy *(Volcheck, 2009).*

![Figure 9: Cladosporium](image)

*Penicillium* species

Species belonging to the genus *Penicillium* are prevalent indoor fungi. Inhalation of *Penicillium* spores in quantities comparable with those encountered by natural exposure can induce both immediate and late asthma in sensitive persons. Among more than 100 different *Penicillium* species, *Penicillium citrinum*, together with *Penicillium chrysogenum* (*Penicillium notatum*), *Penicillium oxalicum*, *Penicillium brevicompactum*, and *Penicillium spinulosum*, were the five most frequently recovered species of *Penicillium* in the United States, whereas *P. citrinum* was the most prevalent *Penicillium* species reported from Taiwan *(Muilenberg et al., 1990).*
Approximately 12 antigens from *P. citrinum* and 11 antigens from *P. chrysogenum* have been shown to react with IgE from patients’ sera by immunoblotting. Several *Penicillium* allergens have also been characterized at the molecular level. Among the *Penicillium* allergens, the 32 to 34 kDa alkaline and/or vacuolar serine proteases were identified as the major allergens of *P. citrinum*, *P. brevicompactum*, *P. chrysogenum*, and *P. oxalicum* (Shen et al., 1999). Immunoblotting data showed that IgE antibodies against components of these prevalent *Penicillium* species could be detected in the sera of approximately 16% to 26% of the asthmatic patients.

Besides the serine protease allergens, a 68-kDa allergen *N*-acetylglucosaminidase and an allergenic heat shock protein belonging to the hsp 70 family have also been identified from *P. chrysogenum* and *P. citrinum*, respectively. The Allergen Nomenclature Subcommittee has designated them *Pen ch 20* and *Pen c 19*, respectively (Shen et al., 1997).

![Figure 10: Penicillium citrinum- distinguishing features of Penicillium 'species' much more evident at 400X. Typical "fingers" made up of the metulae and phialide structures from which chains of conidia extend.](image)

d. **Basidiomycetes**

Basidiomycetes comprise the physically largest and most complex fungi. These include mushrooms, puffballs, rusts, and smuts. Despite being implicated as allergenic fungi, their allergens are not well characterized. *Ganoderma* has been widely studied as wood-decaying allergenic fungi. These fungi produce large shelf-like fruiting bodies called rackets or conks. IgE-binding bands are mostly 18–82 kDa, but no purified antigens have been obtained (Volcheck, 2009).
Other Fungi

Although several fungi have been implicated in allergic disorders, they have not been characterized further to define reliable antigens. Other deuteromycetes that have commonly been implicated include *Helminthosporium*, *Fusarium*, *Aureobasidium (Pullaria)*, *Curvularia*, and *Stemphylium*. *Helminthosporium* and *Fusarium* are field fungi that propagate in the field and release their spores when the soil is disturbed. *Aureobasidium (Pullaria)* is non fermentative yeast that requires a sugary fluid for its substrate, primarily leaves. This is one of the wet-weather spores with counts that increase with the rain. Other wet-weather spores include *Trichoderma* and *Phoma* (Volcheck, 2009).
CHAPTER FIVE

Fungal allergy in respiratory allergic diseases
Fungal allergy in respiratory allergic diseases

Spectrum of respiratory fungal allergic diseases:

The European Academy of Allergy and Clinical Immunology (EAACI) Asthma Section decided to address this deficiency by forming a Task Force to address ‘Fungal Allergy in Asthma’. The first product of the Task Force is a summary of what is and what is not known about this topic, written from the perspective of the progressive practicing clinician (Denning et al., 2014).

Fungi can cause a number of different types of infection, separately from their ability to act as sensitizers. Contrasting patterns of fungi-host interactions are found, with A. fumigatus remarkable as it can cause invasive infection in the immunocompromised, chronic pulmonary aspergillosis and Aspergillus bronchitis in non-immunocompromised individuals with underlying lung damage, and allergic disease of the upper and lower airways. In contrast, Pneumocystis is a pathogen of the immunocompromised, but not an allergenic fungus or sensitizer, which greatly differs from the primary skin pathogen Trichopyton interdigitale (and other species) which causes cutaneous infection in hundreds of millions of people, cannot grow in the lung, but is a common sensitizing fungus associated with severe asthma (Denning et al., 2014).

Broadly speaking, fungi can cause problems to the lung in two ways; either by acting as aeroallergens or as a pathogen causing infection. Some fungi can do both, often simultaneously. Fungal allergens, which can cause rhinitis and asthma, but rarely cause infection, include spores from the plant pathogens Cladosporium and Alternaria spp (Denning et al., 2014).

Allergic bronchopulmonary aspergillosis (ABPA) is an allergic pulmonary disorder caused by hypersensitivity to Aspergillus fumigates clinically manifesting as chronic asthma, recurrent pulmonary infiltrates, and bronchiectasis. The disorder was first described by Hinson et al. in 1952 in the United Kingdom (Hinson et al., 1952).

The condition remains under diagnosed in many countries with reports of mean diagnostic latency of even 10 years between the occurrence of symptoms and the diagnosis. There has been an increase in the number of cases of ABPA due to the heightened physician awareness and the widespread availability of serologic assays (Agarwal, 2009).

The Rosenberg-Patterson criteria are most often used for the diagnosis. There are also a set of minimal diagnostic criteria for ABPA. These criteria continue to be
challenged and modified because there is lack of evidence on the number of criteria that should be present to make the diagnosis. The differentiation of patients with ABPA from patients with Aspergillus hypersensitivity (AH) can also be problematic (Rosenberg, 1977, Patterson, 1986).

Rosenberg-Patterson criteria include Major and minor criteria with major consists of Asthma; Roentgenographic fleeting pulmonary opacities; Skin test positive for Aspergillus (type I reaction, immediate cutaneous hyperreactivity); Eosinophilia; Precipitating antibodies (IgG) in serum; IgE in serum elevated (1,000 IU/mL); Central bronchiectasis; Serums *A. fumigatus-*specific IgG and IgE (more than twice the value of pooled serum samples from patients with asthma who have Aspergillus hypersensitivity) and Minor criteria consists of Presence of Aspergillus in sputum; Expectoration of brownish black mucus plugs; Delayed skin reaction to Aspergillus antigen (type III reaction). The presence of six of eight major criteria makes the diagnosis almost certain; the disease is further classified as ABPA-S that refers to ABPA diagnosed serologically or ABPA-CB that refers to patients with central bronchiectasis on the absence or presence of central bronchiectasis, respectively (Rosenberg, 1977, Patterson, 1986).

A wide spectrum of radiographic appearances can occur in ABPA. The chest radiographic findings of ABPA include transient or fixed pulmonary opacities, tramline shadows, finger-in-glove opacities, and toothpaste shadows. Findings noted on high-resolution CT (HRCT) include central bronchiectasis, mucoid impaction, mosaic attenuation, presence of centrilobular nodules, and tree-in-bud opacities. High-attenuation mucoid impaction (mucus visually denser than the paraspinal muscle) is a pathognomonic finding encountered in patients with ABPA. Central bronchiectasis with peripheral tapering of bronchi on HRCT is believed to be a *sine qua non* for the diagnosis of ABPA (Agarwal, 2011).

The management of ABPA includes two important aspects: institution of glucocorticoids to control the immunologic activity and close monitoring for detection of relapses. Another possible target is the use of antifungal agents to attenuate the fungal burden secondary to the fungal colonization in the airways. It is believed currently that itraconazole can be used only after the first relapse of ABPA despite glucocorticoid therapy or in patients with glucocorticoid dependent ABPA (Agarwal, 2009).

It has been recognized that other fungi can induce a disease process similar to ABPA, normally called allergic bronchopulmonary mycosis (ABPM). Many different fungi can be implicated and the remarkable feature in all is a very high total and specific serum IgE to one or more non-Aspergillus fungi, and other findings usually found in ABPA. Candida albicans is the most commonly reported associated fungus (Chowdhary, 2014).
In 2006, the term severe asthma with fungal sensitization (SAFS) was introduced and subsequently shown to be responsive to antifungal therapy. Most patients with asthma can be well-controlled with low doses of anti-inflammatory agents. However, up to 10% of patients with asthma manifest with severe disease, which leads to considerable limitation of activities of daily life and can be fatal (Denning, 2006).

Fungi can be linked to severe asthma in a multitude of ways, including 1) inhalation of fungal spores; 2) fungal sensitization; and 3) allergic bronchopulmonary aspergillosis (ABPA) (Agarwal, 2011). However, the relationship between severe asthma and fungal sensitization is not easily understood. It is hypothesized that low levels of colonization or direct external exposure to fungi are sufficient to incite an allergic response and bronchial/pulmonary inflammation. The first evidence of the link between fungal sensitization and asthma was published in 1978, when Aspergillus spp sensitization was found to be related to the severity of airway obstruction in 193 asthmatics (Schwartz, 1978). Whether all the fungi really cause SAFS or it is just a casual association remains unknown, as most of these fungi are rarely capable of growth in the human host.

Allergic bronchopulmonary aspergillosis (ABPA) is believed to develop in asthmatics with genetic predisposition. Also SAFS, which is closely related to ABPA, could have a genetic predisposition (Agarwal, 2011).

Severe asthma with fungal sensitization (SAFS) as an entity has only been described recently; hence, a need exists for more data regarding its prevalence, natural history, and clinical relevance to clarify its exact characterization and importance as a specific phenotype of asthma. SAFS is currently more of a diagnosis of exclusion. The criteria proposed for SAFS include the following: 1) severe asthma, 2) evidence of fungal sensitization, and 3) exclusion of ABPA. Exclusion of Allergic Bronchopulmonary Aspergillosis is the most crucial and most difficult step in the diagnosis of SAFS. ABPA is currently believed to be an exaggerated form of Aspergillus sensitization, and is probably the first step in its development (Agarwal, 2011).

The diagnosis of severe asthma is based on the combination of clinical and physiologic severity of the disease. Although many grading systems exist such that proposed in British guideline on the management of asthma in 2008 and National Asthma Education and Prevention Program in 2007, the criteria proposed by the American Thoracic Society remains the most comprehensive and clinically relevant. Basically, a patient with severe asthma is the one who is symptomatic despite use of high doses of inhaled corticosteroids (ICS) and long-acting β2 agonists (LABAs), and requires frequent or continuous doses of oral glucocorticoids for relief of symptoms (Agarwal, 2011).

Patients with asthma become sensitized to fungal antigens, and this subset of asthmatic disease can be termed asthma associated with fungal sensitization. Repeated
exposure causes worsening of asthma control, and these patients are then classified as having SAFS. Patients with asthma associated with fungal sensitization/SAFS go on to develop ABPA due to increasing Th2 immune responses. ABPA usually presents with central bronchiectasis (CB) on CT chest. However, there are patients who fulfill all diagnostic criteria but lack abnormalities on CT chest; they are labeled as seropositive ABPA (ABPA-S). The initial management of SAFS should be akin to that of severe asthma, as both are primarily immune-mediated inflammatory phenomena. Omalizumab and itraconazole should be considered when the response is partial or lacking (Greenberger, 1993).

The benefit of itraconazole in ABPA led to the hypothesis that antifungal azoles also could be useful in the treatment of SAFS. Furthermore, the demonstration of efficacy of antifungal therapy for asthma could also suggest a role for fungal exposure in the pathogenesis of asthma. Only a few studies have used azoles in patients with SAFS. Hence, it is not definite that it was the antifungal action of azole or the anti-inflammatory property of the drug that led to improvement in patients with SAFS (Denning, 2009).

Itraconazole also has been investigated for its use in SAFS in the recently published Fungal Asthma Sensitization Trial (FAST). This was the first study to investigate the role of itraconazole in SAFS due to a variety of fungi. FAST was a randomized, placebo-controlled study that evaluated the effects of 32 weeks of therapy with itraconazole, 200 mg twice daily, in individuals with SAFS. In this study, the effects of itraconazole lasted only for the duration for which it was administered. Although the authors have used itraconazole for 32 weeks, it is currently not clear what should be the optimal dose and duration of azole therapy in SAFS (Denning, 2009).
Fungal sensitization is also found in allergic rhinosinusitis, without asthma or ABPA, and is a diagnostic criterion for allergic Aspergillus rhinosinusitis. Allergic fungal sinusitis (AFS) is a noninvasive form of fungal rhinosinusitis with an incidence of between 6 and 9% of all rhinosinusitis requiring surgery. Regional variation in incidence has been reported, with the southern and southwestern US particularly endemic. Patients with AFS commonly present with chronic rhinosinusitis with nasal polyps, inhalant atopy, elevated total serum immunoglobulin E (IgE), and sinus-obstructing inspissates of a characteristic extramucosal ‘peanut buttery’ visco-elastic eosinophil-rich material called ‘allergic mucin’ that contains sparse numbers of fungal hyphae (Schubert, 2009).

Katzenstein et al showed earlier studies in clinical and pathologic features of seven cases of a newly recognized form of chronic sinusitis are described. AFS shares similar histopathologic features with allergic bronchopulmonary aspergillosis (ABPA) but affects the paranasal sinuses rather than the lung (Katzenstein et al., 1983).

Recently over 10% of patients with chronic obstructive airways disease (COPD) were found to be sensitized to A. fumigatus and this was associated with worse pulmonary function (Bafadhel, 2014).

Another clinical entity, widely acknowledged in several southern countries, is “Trichophyton Asthma”. There are multiple lines of evidence for sensitization to Trichophyton proteins in asthma patients, where inhalation and/or dermal absorption of Trichophyton antigens are currently considered as possible routes of exposure (Ward, 1989).
The phenomenon of Trichophyton asthma has been confirmed in work from several countries including Venezuela, Turkey and, now, Japan. These articles have included evidence that both lungs and nose can be specifically sensitized although this fungus only colonizes the nails and skin; however, the clinical entity is not widely recognized, for several possible reasons. First, the condition is not very common; second, it generally requires intradermal skin tests to demonstrate sensitization, owing to the low quality of fungal extracts; and third, because physicians are surprisingly reluctant to prescribe systemic antifungal treatment (Platts-Mills, 2009).

Mould sensitivity and respiratory allergy severity

In the 1870s, Charles Blackley, a Manchester physician, induced hoarseness, aphonia and an attack of “bronchial catarrh” by inhaling fungi from straw bristle mould (Chaetomium elatum) and Penicillium glaucum (Blackley, 1873).

A remarkable frequency (50%) of mould skin sensitivity principally to Mucor, Penicillium and Aspergillus spp. was then described in Dutch asthmatics (Van Leeuwen, 1925). In 1928, in Germany, Hansen K. found 15% of his asthmatic patients had positive skin tests to Aspergillus or Penicillium and that inhalation challenge reproduced symptoms (Hansen, 1928). In Spain, Jimenez-Diaz C. et al. demonstrated that “house dust” sensitivity was often due to moulds (Jimenez-Diaz, 1932).

Severity of asthma was strongly linked to Alternaria skin-test positivity in the Tucson Cohort, where house dust mite allergy is uncommon (Martinez FD., 1970). No such association was found for pollens or cats, although sensitization to house dust mite was slightly more frequent in those with severe asthma i.e. an excess of house dust mite (D. pteronyssinus) sensitization had also expected in patients with more severe asthma (Peat, 1993). However, reactivity to this allergen was common in all asthma groups and only slightly commoner in patients with multiple admissions (Zureik, 2002).

Asthma deaths, hospital admissions, respiratory symptoms and peak expiratory flow rates can be adversely affected by high fungal spore concentrations in outdoor air (Nelson et al., 1999). The seasonal (summer–autumn) peak of asthma admissions occurs when ambient air counts of moulds are high, in contrast to snowy conditions when spore counts are very low (Jenkins, 1981).

Sara Downs et al. reported that the severity of airway hyperresponsiveness, which is a fundamental component of current symptomatic asthma, increased with airborne Alternaria spore concentrations and that the effect was significantly greater in Alternaria-sensitized children. They also found that wheeze increased in periods of high
spore concentrations, although this effect was not specific for children sensitized to *Alternaria*. Standard methods were used to measure allergic sensitization, symptoms, medication use, and airway responsiveness and Airway hyperresponsiveness is a highly specific indicator for asthma and asthma exacerbations, airway inflammation and long-term asthma (*Downs et al.*, 2001).

Mahmoud Zureik et al conclude that sensitization to moulds might be involved in the severity of asthma. It was a cross-sectional study of 1132 adults with asthma found that sensitization to *Alternaria* or *Cladosporium* is a powerful risk factor for severe asthma in several European countries. Given the increase of asthma and the prevalence of severe asthma in the past decades these results may be relevant for many people (*Zureik et al.*, 2002).

Black et al. found that subjects who had been admitted to ICU with severe, potentially life-threatening asthma were significantly more likely to have one or more positive skin tests for fungal dry weather spores. Indeed, positive skin tests for fungi occurred almost twice as frequently in the subjects in ICU group. This difference does not reflect an increase in sensitization to all inhalant allergens. Patients admitted to the ICU were no more likely to have positive skin tests to grass pollen, cat dander, or *D. pteronyssinus* than the other subjects (*Black et al.*, 2000).

Boulet et al. paper showed that in subjects with respiratory allergic symptoms, indoor allergen sensitization is strongly associated with asthma, while exclusive sensitization to pollens is associated primarily with rhinitis. Sensitization was more prevalent for indoor allergens than for outdoor allergens in all groups determined according to diagnosis or age. Indices of atopy were higher in men in the group >18 years old. Prevalence and degree of sensitization were shown to peak in young adults, regardless of the allergen, and to diminish with age. This study stresses the role of indoor allergens in the development of asthma and shows the variability of allergic manifestations according to the type of sensitization (*Boulet et al.*, 1997).

O'Driscoll et al. stated that mould (and dog) sensitization is common in patients with severe asthma requiring multiple hospital admissions in Manchester (*O'Driscoll et al.*, 2005). These observations beg the question of why sensitization to fungal spores is a risk factor for life-threatening attacks. Fungal spores are smaller in size than pollen grains, and this fact will favors deposition of spores in the airways (*Solomon, 1993*). Bronchial provocation tests performed with *Alternaria* are more likely to produce late asthmatic responses than those performed with grass pollen (*Richerson*, 1986). In addition, the spore counts in the atmosphere are usually many times higher than pollen counts. Certainly, spore counts for *Alternaria* and *Cladosporium* and other fungi can fluctuate dramatically from one day to another (*Brown, 1978*).
Mechanisms for fungus related respiratory allergic diseases.

Exposure to fungal biomass consists of exposure to living fungal spores and fungal debris after death of fungal spores and mycelium. Fungal spores generally do not easily release their allergen content, owing to the rigidity of the spore wall, and release of allergens will mainly occur during germination of the spores (Mitakakis et al., 2001) or after cell death. Germination times of spores differ largely, showing a short germination time of 2 hours for *Alternaria* compared with the germination time of other fungal spores (germination time 5–10 hours) (Takatori et al., 1994). Aeroallergens in death fungal masses are more soluble and readily available for contact with the airway system, which most likely explains the rate of asthmatic attacks (often within 10–15 minutes) after inhalation of mould-contaminated air (eg, in barns) by sensitized patients (Kauffman, 2003).

Fungal biomass that is deposited on the airway wall is eliminated by the combined action of innate recognition by complement and surfactant proteins that facilitate phagocytosis by alveolar macrophages. Additionally, secretory immunoglobulin A (S-IgA) prevents contact with the epithelial cell layer and allows transport on the epithelial lining fluid (ELF) by coordinated ciliary movement to the oropharyngeal cavity (Kauffman, 1999). In healthy airways, this transport will generally remove particles impacted in the larger airways in approximately 4 hours, before germination of fungal spores can occur. Smaller-sized spores (*Aspergillus* and *Penicillium*) can reach the lower airways and can be removed in 12 to 24 hours (Takatori et al., 1994).

Spores that are deposited in large quantities and allergens from dead fungal mass might reach the epithelial cell surface and activate epithelial cells. Epithelial cells are equipped with various receptors, such as Toll-like receptors (TLR) that can recognize surface structures of microorganisms, the so-called pathogen-associated molecular patterns (PAMP). In addition to activation by TLRs, proteolytic components released by fungi can interact with the epithelial cell surface by disruption of cellular junctional contacts and activation of so called protease-activated receptors (PAR) that will induce the production of cytokines and prostaglandin (PG) E2 (Medzhitov et al., 2000).

Fungal extracts have shown differences in their capacity to cause protease-dependent disruption of cellular contacts and release chemokines. Extracts of *Alternaria* were more active in desquamation and proteinase-dependent activation of epithelial cells, whereas *Cladosporium* did not show desquamation and much less activation (Kauffman et al., 2000). It has been proposed that proteases present in inhaled allergens (including fungi) affecting epithelial cellular contacts might facilitate passage of allergens through the epithelial barrier. Activation of TLRs and PARs on epithelial cells, followed by facilitated transport of allergens and production of cytokines and growth factors, might
be involved in increased sensitization to (fungal) allergens and inflammatory response and remodeling of the airways (Kauffman, 2003).

Research on the genetics of asthma indicates two types of genetic predisposition; genes related to formation of IgE and genes related to development of AHR, which interact to determine the final phenotype of atopic asthma (Kauffman et al., 2003).

The proteases present in HDMs "House Dust Mites" (especially the cysteine proteinase Der p1) are most potent in breaching the resistance of the epithelial cell layer, facilitating the Th2-type immune response to HDM allergens. Therefore, the association of sensitization to HDMs and asthma will be found in the full range from mild to severe asthma. Fungal asthma is induced by high exposure to allergen, and the proteases involved are less active. Therefore, fungus-induced asthma is found in patients with a high susceptibility of airways for environmental stimuli, which is determined by more cumulative interactions of genes for AHR and/or atopy. This hypothesis might explain why fungus-induced asthma is mainly found and associated with more severe asthma (Kauffman et al., 2003).

Nearly all house dust contains fungal spores and mycelia fragments, which may easily become airborne upon disturbance (eg, walking across carpet). Indoor exposures to fungi are of particular interest due to the increased tendency for people to spend time inside buildings constructed for energy efficiency and therefore with limited ventilation (Chiu et al., 2007).

Nearly all fungal allergens identified thus far are proteins, many of which have enzyme activity. There has been much speculation that enzymes, particularly proteolytic enzymes, may serve to enhance the allergenicity of these proteins. Because proteolytic enzymes by their nature must be able to resist self-destruction, they tend to persist in the environment. Pen ch 13, for example, is a serine protease that is one of the major allergens produced by Penicillium citrinum. This enzyme has been found to dose dependently induce interleukin (IL)-8 cytokine expression in airway epithelial cells. Pen ch 13 also cleaves protease activated receptors at their activation sites and induces intracellular Ca\(^{+2}\) mobilization. All these actions tend to steer the immune response toward a T helper (Th) 2 phenotype (Chiu et al., 2007).

Fungi produce several potentially irritating substances. These include microbially derived volatile organic compounds (VOCs), glucans (which are related to endotoxins), and ergosterols, although the full spectrum of fungal irritants has not been fully enumerated. As with other irritants, the health effects of exposure to these substances is directly related to the amount and duration of exposure. That is why well-ventilated buildings are considered to be healthier than those in which airborne potentially respirable irritants can accumulate (Portnoy et al., 2008).
Because many substances in buildings are volatile and potentially irritating, it often is difficult to determine whether a particular inflammatory reaction is due to irritants derived from fungi or from other sources. Therefore, to document that a fungally derived substance is responsible for a symptom, it is necessary to demonstrate that the substance is present in the environment, exposure is of sufficient magnitude and duration to trigger the observed reaction in the affected individual, and other substances that could account for the symptoms are not present (Portnoy et al., 2008).

Ergosterol and glucans are fungal-derived substances that correlate with total fungal biomass (Schnurer, 1993). The β (1-3) D glucan from fungal cell walls is suspected to produce nonspecific inflammation due to its attachment to lipopolysaccharide binding protein, CD14 cell surface protein, and Toll-like receptor (TLR)-4. This binding leads to production of local inflammatory cytokines that result in the migration of inflammatory cells into the lung. These cytokines may also penetrate into the blood and attract additional inflammatory cells (Portnoy et al., 2008).

Mycotoxins are organic compounds of small molecular weight that, although generally not volatile, often attach to small particles that can become airborne. Therefore, enumeration of airborne spores may not account for total mycotoxin exposure any more than cat hair or dust mite fecal pellet counts would accurately describe cat or dust mite allergen exposure. Many but not all fungi species produce mycotoxins. Toxigenic fungi vary in the amount and types of mycotoxins produced, depending on the substrate on which they grow and other environmental conditions. The toxic effects of airborne mycotoxins and other irritants as they relate to asthma are poorly understood (El-Maghraby, 1991).

Two types of evidence have been used to support the hypothesis that exposure to fungi can trigger asthma symptoms in persons already known to have asthma. These include demonstration of a direct correlation between the development of asthma symptoms and exposure to fungi, and observations that high exposure to fungi (e.g. during thunderstorms) corresponds to emergency department visits and hospitalization for treatment of asthma episodes. Though it is reasonable to expect that exposure to fungi in sensitized individuals with asthma is likely to lead to symptoms, it is less clear that exposure to fungi is likely to increase the risk of developing asthma. The problem is that the cause of asthma itself is not known. Until this question is resolved, the best we can expect from studies of fungi and asthma is circumstantial evidence of increased exposure in individuals who develop asthma (Portnoy et al., 2008).

Evidence for an association between the presence of fungi and the genesis of asthma is more tentative than the evidence for fungal spores as asthma triggers. Belanger et al. studied 849 newborn infants with an asthmatic sibling during their first year of life, and found that persistent exposure to fungi increased the risk of wheezing both in infants of
mothers who had asthma and in infants of mothers without asthma (Belanger et al., 2003).
PATIENTS AND METHODS
Patients and methods

Study design

This cross-sectional study was conducted at Allergy Outpatient Clinic of Ain Shams University Hospital during the period from February 2015 to June 2016, included 200 patients who were diagnosed with allergic bronchial asthma and/or allergic rhinitis. All cases were selected from patients who were followed up at Allergy Outpatient Clinic of Ain Shams University Hospital.

Allergic bronchial asthma was diagnosed according to the guidelines of the 2015 GINA Report, Global Strategy for Asthma Management and Prevention (GINA, 2015); the Criteria used for diagnosis of asthma included a history of variable respiratory symptoms and evidence of variable expiratory airflow limitation as follows;

A. Variable respiratory symptoms used in diagnosis of allergic bronchial asthma:

Typical symptoms include wheeze, shortness of breath, chest tightness, and cough; asthmatic patients generally have more than one of these symptoms. The symptoms occur variably over time and vary in intensity; also the symptoms often occur or become worse at night or on walking. Symptoms are often triggered by exercise, laughter, allergens or cold air. Symptoms often occur with or worsen with viral infections.

B. Evidence of variable expiratory airflow limitation:

At least once during the diagnostic process when FEV1 is low, document that the FEV1 / FVC ratio is reduced below 0.80 (normally more than 0.75-0.80 and more than 0.90 in children).

Document that variation in lung function is greater than in healthy people, (one or more test below):

FEV1 increases by more than 12% and 200 ml of initial FEV1 (in children, ≥12% of the predicted value) after inhaling a bronchodilator, this is called bronchodilator reversibility.

Average daily diurnal PEF variability is ≥ 10 % (in children, ≥ 13%)

FEV1 increase by more than 12 % and 200 ml from baseline (in children by ≥ 12% of the predicted value) after 4 weeks of anti-inflammatory treatment (outside respiratory infection)
In the selected patients, a history of variable respiratory symptoms that discussed above was found, and the selected patients with asthma generally have more than one of these symptoms in addition to evidence of variable expiratory airflow limitation. The selected patient had low FEV1, document that FEV1 / FVC ratio was reduced with positive bronchodilator reversibility test (short acting beta agonist inhaler "SABA" has been withholded 4 hours before the test and long acting beta agonist inhaler "LABA" was withholded 12 hours before the test). Choose of spirometry with bronchodilator reversibility as a surrogate for variation of lung function was used due to its feasibility and easy for patients to perform.

Allergic rhinitis patients were diagnosed by the presence of recurrent episodes of runny nose, sneezing, nasal congestion, and itching, according to the most recent Allergic Rhinitis and its Impact on Asthma guidelines (ARIA) (Bousquet et al., 2008). Allergic rhinitis patients were differentiated from nonallergic rhinitis patients and chronic sinusitis by typical signs and symptoms of clear rhinorrhea, sneezing, congestion and pruritus of nose, eye, oral mucosa with positive past history and/or family history allergic disease, temporal association with allergic aeroallergens.

An informed consent was taken from all studied participants after ensuring the data confidentiality. The study was approved by the Institutional Ethics Committee of the Faculty of Medicine, Benha University and Ain Shams University Hospital.

**Selection criteria:**

Our Patients were selected from those who enrolled for follow-up at Allergy Outpatient Clinic of Ain Shams University Hospital. The selected patients should fulfill the diagnostic criteria of allergic rhinitis and / or allergic bronchial asthma that mentioned before.

According to the guidelines of the 2015 GINA Report, Global Strategy for Asthma Management and Prevention, allergic bronchial asthma phenotype should to be commenced in childhood and were associated with a past and/or family history of allergic diseases such as allergic rhinitis, food allergy, etc. Allergic rhinitis patients were selected, as well as cases with both allergic bronchial asthma and allergic rhinitis. Both males and females were included in the study; patients with various body weights are included; and various age groups were studied. In allergic bronchial asthma, all levels of control were selected. In allergic rhinitis all grades of severity whether mild and moderate-to-severe were included in the study (GINA, 2015).
Exclusion criteria:

Immunocompromised patients such as those having malignancy or immunodeficiency state were excluded from the study as it will be hazardous on patient if the rare side effect of anaphylaxis related to skin prick test occurred also patients on antihistamine therapy and other drugs that affect skin prick test reproducibility within a week before enrollment were excluded from study temporarily during this period. Patients on beta blockers agents were excluded from study due its hazardous effect while performing SPT

Pregnant women were excluded from study owing to risk of anaphylaxis when performing skin prick test and it may be hazardous on mother and fetus although it is a rare incidence. Patients with chronic respiratory tract infections were excluded; also those who have chronic obstructive airway disease were omitted from study.

Methods

For each patient detailed history was obtained including age, sex, occupation; also history included an evaluation of nature, duration, and time course of symptoms; possible triggers for symptoms; response to medications; co-morbid conditions; family history of allergic diseases; environmental exposures; occupational exposures; and effects on quality of life. A thorough history may help identify specific triggers, suggesting an allergic etiology for the rhinitis and asthma. Mould exposure and dampness in home and workplace was asked about.

Physical examination stressed on chest examination, including respiratory rate, shape of the chest, auscultation of breath sounds and additional sounds if found. Ear, Nose and Throat (ENT) examination was also performed. Skin examination was mandatory to guarantee that extensive eczema, dermatographism was not found as it may affect reproducibility of skin prick test

Assessment of asthma control level, including a questionnaire about the frequency of daytime symptoms, nocturnal awakenings, limitation of activities, and need for rescue medications. This was based on criteria established by 2015 GINA Report, Global Strategy for Asthma Management and Prevention. In the past 4 weeks, has the patient had: Daytime symptoms more than twice/week? ; Any night waking due to asthma? ; Reliever needed more than twice /week? ; Any activity limitation due to asthma?. Based on answering these questions, the patients were categorized into well-controlled asthma (when none of these was found), partially controlled asthma (when 1-2 of these were found) and uncontrolled asthma (when 3-4 of these were found).

The severity of allergic rhinitis has been classified as "mild" and "moderate/severe" depending on the severity of symptoms and quality-of-life outcomes. The "Mild" allergic rhinitis means that none of the following 4 items are
present: Sleep disturbance; Impairment of daily activities, leisure and/or sport; Impairment of school or work; presence of symptoms but not troublesome. The "Moderate/severe" Allergic rhinitis means presence of one or more of the following four items: Sleep disturbance; Impairment of daily activities, leisure and/or sport; Impairment of school or work and troublesome symptoms (Bousquet et al., 2008).

For each patient, two mL of whole blood was withdrawn and placed in a test tube containing EDTA to prevent clotting, and complete blood count with special emphasis on the presence of eosinophilia percentage were performed by an automated analyzer.

Spirometry was done for measurement of forced expiratory volume in 1st second (FEV1), FEV1/FVC and bronchodilator reversibility. This was carried out at the Pulmonary Functions Laboratory unit at Ain Shams University Hospital.

For each patient, skin prick test to mould allergens beside other common aeroallergens using commercial standardized extracts were done.

Skin test procedure:

Briefly, the skin of anterior surface of the right forearm will be cleaned, sterilized, and one drop of each allergen extract was carefully applied approximately 3 cm apart. They should not be placed closer than 5 cm to the wrist or less than 3 cm from the elbow crease as sensitivity to skin testing varies two-fold between the elbow and wrist. The epidermis was pricked carefully by a lancet through each allergen extract drop, without causing bleeding. After 20 minutes, the mean wheal diameter was measured by summation the largest diameter to the diameter perpendicular to it, and dividing the result by two. Histamine (0.1%) in HCL-buffered saline and physiologic saline was used as positive and negative controls, respectively (Heinzerling et al., 2013).

The skin prick test (SPT) was considered valid if the difference in mean wheal diameter between the positive and negative controls is at least 1 mm. A mean wheal diameter of at least 3 mm greater than the negative control will be considered positive (Heinzerling et al., 2013).

All extracts for skin testing were supplied in early 2015 by Stallergenes (Antony, Hauts-de-Seine, France) company in France. The test was conducted to all selected patients. While performing the tests, the used allergen extracts were frequently checked for expiratory date, and included the following standardized extracts:
Fungal allergens:

- Alternaria alternata
- Chaetomium globosum
- Helminthosporium halodes
- Pullularia pullulans (dimorphic fungi)
- Aspergillus mix (A. fumigatus, A. nidulans, A. niger)
- Cladosporium mix (C. cladosporioides, C. herbarum)
- Penicillium mix (P. digitatum, P. expansum, P. notatum)
- Mucor racemosus (dimorphic fungi)
- Rhizopus nigricans
- Merulius lacrymans

Non fungal aeroallergens:

- Betulaceae tree mixtures
- Chenopodiaceae weed mixtures
- Grasses mixture.
- Dermatophagoides pteronyssinus
- Dermatophagoides farina
- Cat epithelium
**Statistical methods:**

The collected data were tabulated and analyzed using statistical package of social sciences SPSS version 20 software (Spss Inc, Chicago, ILL Company). Categorical data were presented as number and percentages while quantitative data were expressed as mean ±standard deviation. Fisher's exact test and chi square test were used to compare different groups. The accepted level of significance in this work was stated at 0.05 so P-value <0.05 was considered significant, P-value >0.05 was insignificant and P-value <0.001 was highly significant.
RESULTS
Results

This study was conducted on 200 patients with allergic bronchial asthma and/or allergic rhinitis who selected from those followed-up at the Allergy and Clinical Immunology Clinic at Ain Shams University Hospitals. The studied patients included 70 males (35%) and 130 females (65%). The study included various age groups, ranging from 6 years old to 70 years old, with a mean age of 32.2 ± 14.4 years. Out of studied patients, 28 (14%) were aged from 6 to 14 years old, 132 (66%) were aged from 15 to 44 years and the remaining 40 patients (20%) were aged from 45 to 70 years (table 1 and 2).

Out of studied patients, 40% (80 patients) had Allergic asthma, 47% (94 patients) had allergic rhinitis and the remaining 13% (26 patients) had combined allergic bronchial asthma and allergic rhinitis (table 3 and figure 1).

Our study revealed that 106 patients (53% of studied patients) had allergic bronchial asthma, of whom 80 patients (75.5%) had only allergic bronchial asthma and 26 patients (24.5%) had allergic bronchial asthma in combination with allergic rhinitis (figure 2). On the other hand, 120 patients (60% of studied patients) had allergic rhinitis, of whom, 94 patients (78.3%) had only allergic rhinitis and 26 patients (21.7%) had allergic rhinitis in combination with allergic bronchial asthma (figure 3).

Regarding the degree of severity of allergic rhinitis, based on ARIA questionnaire, there were 34 patients (28.3%) had mild allergic rhinitis (table 4). On the other hand, 86 patients (71.7%) had moderate to severe allergic rhinitis (table 4).

Regarding the control state of allergic bronchial asthma patients and combined allergic rhinitis with allergic asthma who were 106 patients, there were 26 patients (24.5%) were well controlled, whereas 62 patients (58.5%) were partially controlled and 18 patients (17%) were poorly controlled (table 5 and figure 4).

The results of skin prick test to all studied patients revealed that 74% (148 out of all studied patients) had positive results to at least one allergen extract and only 26% (52 patients out of all studied patients) had negative skin prick test (table 6 and figure 5).

Out of 148 patients who showed positivity to at least one allergen extract, 62 patients (41.9%) showed positivity towards fungal allergen. Out of the 62 patients who showed positivity towards fungal extract, there were 38 (25.7%) patients showed positivity towards only fungal allergen extracts and 24 (16.2%) patients who showed positivity towards fungal and nonfungal allergen extracts. On the other side, 86 patients out of all positive skin prick test patients (58.1%) were positive towards only non fungal allergens (table 7).
When comparing the incidence of skin prick test results between various age groups, the study showed 28 patients (14 %) had age ranges from 6 years to 14 years (young and older children); of whom, 4 patients showed negative skin prick test, 10 patients showed positive towards only fungal extract, 2 patients showed positive towards both fungal and non fungal extracts and finally 12 patients showed positive towards non fungal allergen extract (table 8).

Out of 132 patients who aged of 15 - 44 years, 40 patients (30.3%) had negative skin prick test, 52 patients (39.4%) had positive results to non fungal extracts and remaining 40 patients (30.3%) had positive results towards fungal extracts; of whom, 22 patients had positive results for only fungal extracts and 18 patients had positive results to combined fungal and non fungal allergens (table 8).

Out of 40 patients who had age range of 45 years to 70 years, 8 patients (20%) were negative skin prick test, 22 patients (55%) were positive towards only non fungal extracts and remaining 10 patients (25%) had positive results for fungal extracts; of whom, 6 patients were positive towards only fungal allergens and 4 patients were positive to combined fungal and non fungal allergens (table 8).

Regarding results of skin prick test to common fungal extracts in the studied positive fungal population, the results showed that *Alternaria alternate* represents 32.2% (20 patients out of 62 patients), *Pencillium mix* also represents 32.2% (20 patients out of 62 patients) of positive fungal sensitivity patients, followed by *Aspergellus mix* and *Chaetomium globosum* each accounting for 22.6% (14 patients from 62 patients) of positive fungal sensitivity patients and then in 5th order was *Mucor racemosus* that was accounting for 19.4% (12 patients from 62 patients) (table 9 and figure 6).

Regarding the results of skin prick test to common non fungal allergen extracts in the studied positive skin prick test population, the study showed that dust mites extracts with its two species *Dermatophagoides farina* and *Dermatophagoides pteronyssinus* sensitivity represent the highest prevalence of allergen extracts sensitivity, accounting for 50 % (74 patients out of 148 patients) and 43.2 % (64 patients out of 148 patients) respectively; then in 3rd order was Cat epithelium sensitivity accounting for 18.9 % (28 patients of 148 patients) (table 10 and figure 7).

In a trial to relate fungal sensitivity with predominant atopic symptoms, the study showed that dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farina*) represent the highest prevalence in all patients with positive skin prick test whether their predominant atopic symptoms were allergic rhinitis, allergic bronchial asthma or both. The study showed that *Dermatophagoides pteronyssinus* accounts for 31.9% in patients with allergic rhinitis (30 patients out of 94 patients), 30% in patients with allergic bronchial asthma (24 patients out of 80 patients) and 38.5% in
patient with combined allergic rhinitis and allergic bronchial asthma (10 out of 26 patients). *Dermatophagoides farina* accounts for 38.3% (36 out of 94 patients) in patients with allergic rhinitis, 36.3% (29 out of 80 patients) in patients with allergic bronchial asthma and 34.6% (9 out of 26 patients) in patients with combined allergic rhinitis and allergic bronchial asthma as shown in (table 10). In patients with allergic rhinitis or allergic bronchial asthma, *Alternaria alternata* and *Pencillium mix* were the second in prevalence after dust mites. In patients with combined allergic rhinitis and allergic bronchial asthma, the results showed that *Pullularia pulluans* and *Merulium lacrymans* represent the highest prevalence after dust mites' sensitivities and these results were significant as P values of *Pullularia pulluans*, *Merulium lacrymans* and *Chenopodiaceae* weed mixture represent (0.006), (0.002), and (0.02) respectively (table 11).

When studying the relation of the level of asthma control (as assessed by GINA 2015 Questionnaire) and fungal sensitivities, the result showed that fungal sensitivities in studied allergic bronchial asthma population were distributed as; 8 out of 26 patients (30.8%) in well controlled patient's category, 19 out of 62 patients (30.6%) of partially controlled category and 7 out of 18 patients (38.9%) of poorly controlled category. This means the percentage of fungal sensitized patients in poorly controlled category was higher than percentage of fungal sensitized patients in well controlled category with P value of 0.02 (table 12).

Also when studying the relation of the level of asthma control (as assessed by GINA 2015 Questionnaire) and the number of positive fungal sensitivity. The results showed that highest percentage (19.2%) of patients in well controlled category were sensitized to only one of studied fungal extracts, whereas, the highest percentage (11.1%) of patients in poorly controlled category were sensitized to 3 of studied fungal extracts (table 12).
Table (1): **Distribution of studied patients regarding various age groups (n=200 patients):**

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>Mean ± SD</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-70 years old</td>
<td>32.2 ± 14.4</td>
<td>200 patients (100%)</td>
</tr>
<tr>
<td>6-14 years old</td>
<td></td>
<td>28 (14%)</td>
</tr>
<tr>
<td>15-44 years old</td>
<td></td>
<td>132 (66%)</td>
</tr>
<tr>
<td>45-70 years old</td>
<td></td>
<td>40 (20%)</td>
</tr>
</tbody>
</table>

Table (2): **Distribution of genders and their ages in studied population (n =200 patients)**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number (%)</th>
<th>Age (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>70 (35%)</td>
<td>24±13.16</td>
</tr>
<tr>
<td>Females</td>
<td>130 (65%)</td>
<td>35.95±13.03</td>
</tr>
</tbody>
</table>

Table (3): **Types of respiratory allergy and their prevalence among studied population (n=200 patients):**

<table>
<thead>
<tr>
<th>Type of respiratory allergy</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic Bronchial asthma</td>
<td>80 (40%)</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>94 (47%)</td>
</tr>
<tr>
<td>Combined allergic bronchial asthma and allergic rhinitis</td>
<td>26 (13%)</td>
</tr>
</tbody>
</table>
Figure (1): Percentage of various types of respiratory allergy in studied population (n= 200 patients).

- Bronchial asthma: 40%
- Allergic rhinitis: 47%
- Combined bronchial asthma and allergic rhinitis: 13%

Figure (2): Percentage of patients with combined allergic bronchial asthma and allergic rhinitis among those with allergic rhinitis (n=120 patients)

- Allergic rhinitis: 78.30%
- Combined B.A & RA: 21.70%
Figure (3): Percentage of patients with combined allergic bronchial asthma and allergic rhinitis among patients with allergic bronchial asthma (n= 106 patients)

Table (4): Categorization of allergic rhinitis patients and allergic rhinitis with allergic bronchial asthma according to severity of rhinitis (n= 120 patients):

<table>
<thead>
<tr>
<th>Allergic rhinitis severity</th>
<th>Number ( % )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>34 (28.3%)</td>
</tr>
<tr>
<td>Moderate to severe</td>
<td>86 (71.7%)</td>
</tr>
</tbody>
</table>
Table (5): Patient's categorization based on the degree of asthma control (n: 106):

<table>
<thead>
<tr>
<th>Degree of control</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>well controlled</td>
<td>26 (24.5%)</td>
</tr>
<tr>
<td>partially controlled</td>
<td>62 (58.5%)</td>
</tr>
<tr>
<td>poorly controlled</td>
<td>18 (17%)</td>
</tr>
</tbody>
</table>

Figure (4): Patient's categorization based on the degree of asthma control (n: 106):
Table (6): Results of skin prick test in studied patients (n = 200 patients)

<table>
<thead>
<tr>
<th>SPT results</th>
<th>Number ( % )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>148 (74%)</td>
</tr>
<tr>
<td>Negative</td>
<td>52 (26%)</td>
</tr>
</tbody>
</table>

Figure (5): Results of skin prick test in studied patients (n = 200 patients)
Table (7): **Positive SPT regarding fungal sensitivity (n = 148 patients)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive towards fungus</td>
<td>62 patients (41.9%)</td>
</tr>
<tr>
<td>Positive towards only fungal allergen extract</td>
<td>38 patients (25.7%)</td>
</tr>
<tr>
<td>Positive towards fungal and non fungal allergen extracts</td>
<td>24 patients (16.2%)</td>
</tr>
<tr>
<td>Positive towards other allergens</td>
<td>86 patients (58.1%)</td>
</tr>
</tbody>
</table>
Table 8: Results of skin prick tests in various age groups of studied patients (n=200 patients):

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number (%)</th>
<th>Number (%)</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>6-14 years old</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive towards fungus</td>
<td>12 patients (42.9%)</td>
<td>Positive towards only fungal allergen extract</td>
<td>10 patients (35.8%)</td>
</tr>
<tr>
<td>Positive towards fungal and non fungal allergen extracts</td>
<td>2 patients (7.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive towards other allergens</td>
<td>12 patients (42.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative skin prick test</td>
<td>4 patients (14.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>15-44 years old</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive towards fungus</td>
<td>40 patients (30.3%)</td>
<td>Positive towards only fungal allergen extract</td>
<td>22 patients (16.7%)</td>
</tr>
<tr>
<td>Positive towards fungal and non fungal allergen extracts</td>
<td>18 patients (13.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive towards other allergens</td>
<td>52 patients (39.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative skin prick test</td>
<td>40 patients (30.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>45-70 years old</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive towards fungus</td>
<td>10 patients (25%)</td>
<td>Positive towards only fungus allergen extract</td>
<td>6 patients (15%)</td>
</tr>
<tr>
<td>Positive towards fungal and non fungal allergen extracts</td>
<td>4 patients (10%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive towards other allergens</td>
<td>22 patients (55%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative skin prick test</td>
<td>8 patients (20%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table (9): Results of SPT to common fungal allergens in studied population (n=62 patients):

<table>
<thead>
<tr>
<th>Positive fungal allergen by SPT</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria alternata</td>
<td>20 (32.2%)</td>
</tr>
<tr>
<td>Penicillium mix</td>
<td>20 (32.2%)</td>
</tr>
<tr>
<td>Aspergillus mix</td>
<td>14 (22.6%)</td>
</tr>
<tr>
<td>Chaetomium globosum</td>
<td>14 (22.6%)</td>
</tr>
<tr>
<td>Mucor racemosus</td>
<td>12 (19.4%)</td>
</tr>
<tr>
<td>Helminthosporium halodes</td>
<td>10 (16.1%)</td>
</tr>
<tr>
<td>Cladosporium mix</td>
<td>10 (16.1%)</td>
</tr>
<tr>
<td>Rhizopus nigricans</td>
<td>10 (16.1%)</td>
</tr>
<tr>
<td>Merulius lacrymans</td>
<td>8 (12.9%)</td>
</tr>
<tr>
<td>Pullularia pullulans</td>
<td>4 (6.5%)</td>
</tr>
</tbody>
</table>

Figure (6): Results of SPT to fungal allergens in studied population (n=62 patients):
Table (10): **Results of SPT to other non-fungal allergens from positive group (n=148):**

<table>
<thead>
<tr>
<th>Non fungal extracts</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermatophagoides farina</td>
<td>74 (50 %)</td>
</tr>
<tr>
<td>Dermatophagoides pteronyssinus</td>
<td>64 (43.2 %)</td>
</tr>
<tr>
<td>Cat epithelium</td>
<td>28 (18.9 %)</td>
</tr>
<tr>
<td>Grasses mixture</td>
<td>12 (8.1 %)</td>
</tr>
<tr>
<td>Chenopodiaceae weed mixtures</td>
<td>1 (6.7 %)</td>
</tr>
<tr>
<td>Betulaceae tree mixtures</td>
<td>4 (2.7 %)</td>
</tr>
</tbody>
</table>

Figure (7): **SPT to other non-fungal allergens**
Table (11): Results of SPT to common allergens in studied patients regarding to predominant atopic symptoms:

<table>
<thead>
<tr>
<th>Positive allergen by SPT</th>
<th>Allergic rhinitis (n=94) N (%)</th>
<th>Allergic Bronchial asthma (n=80) N (%)</th>
<th>Combined Bronchial asthma and allergic rhinitis (n=26) N (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria alternata</td>
<td>10 (10.6%)</td>
<td>9 (11.3%)</td>
<td>1 (3.8%)</td>
<td>0.57</td>
</tr>
<tr>
<td>Chaetomium globosum</td>
<td>6 (6.4%)</td>
<td>7 (8.8%)</td>
<td>1 (3.8%)</td>
<td>0.66</td>
</tr>
<tr>
<td>Helminthosporium halodes</td>
<td>4 (4.3%)</td>
<td>5 (6.3%)</td>
<td>1 (3.8%)</td>
<td>0.89</td>
</tr>
<tr>
<td>Pullularia pullulans</td>
<td>2 (2.1%)</td>
<td>2 (2.5%)</td>
<td>4 (15.4%)</td>
<td>0.006</td>
</tr>
<tr>
<td>Aspergillus mix</td>
<td>7 (7.4%)</td>
<td>7 (8.8%)</td>
<td>0 (0.0%)</td>
<td>0.31</td>
</tr>
<tr>
<td>Cladosporium mix</td>
<td>5 (5.3%)</td>
<td>4 (5%)</td>
<td>1 (3.8%)</td>
<td>0.95</td>
</tr>
<tr>
<td>Penicillium mix</td>
<td>10 (10.6%)</td>
<td>8 (10%)</td>
<td>2 (7.7%)</td>
<td>0.91</td>
</tr>
<tr>
<td>Mucor racemosus</td>
<td>6 (6.4%)</td>
<td>6 (7.5%)</td>
<td>0 (0.0%)</td>
<td>0.37</td>
</tr>
<tr>
<td>Rhizopus nigricans</td>
<td>3 (3.2%)</td>
<td>4 (5%)</td>
<td>2 (7.7%)</td>
<td>0.59</td>
</tr>
<tr>
<td>Merulius lacrymans</td>
<td>4 (4.3%)</td>
<td>0 (0%)</td>
<td>4 (15.4%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Betulaceae tree mixtures</td>
<td>2 (2.1%)</td>
<td>2 (2.5%)</td>
<td>0 (0%)</td>
<td>0.73</td>
</tr>
<tr>
<td>Chenopodiaceae weed mixtures</td>
<td>5 (5.3%)</td>
<td>1 (1.3%)</td>
<td>4 (15.4%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Grasses mixture</td>
<td>6 (6.4%)</td>
<td>6 (7.5%)</td>
<td>0 (0%)</td>
<td>0.37</td>
</tr>
<tr>
<td>Dermatophagoides pteronyssinus</td>
<td>30 (31.9%)</td>
<td>24 (30%)</td>
<td>10 (38.5%)</td>
<td>0.08</td>
</tr>
<tr>
<td>Dermatophagoides farina</td>
<td>36 (38.3%)</td>
<td>29 (36.3%)</td>
<td>9 (34.6%)</td>
<td>0.54</td>
</tr>
<tr>
<td>Cat epithelium</td>
<td>14 (14.9%)</td>
<td>12 (15%)</td>
<td>2 (7.7%)</td>
<td>0.61</td>
</tr>
</tbody>
</table>
Table (12): **Fungal sensitivity and level of asthma control:**

<table>
<thead>
<tr>
<th>Fungal allergens number</th>
<th>Well controlled (26 patients)</th>
<th>Partially controlled (62 patients)</th>
<th>Poorly controlled (18 patients)</th>
<th>$X^2$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5(19.2%)</td>
<td>13(29.9%)</td>
<td>0(0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1(3.8%)</td>
<td>1(1.6%)</td>
<td>4(22.2%)</td>
<td>18.01</td>
<td>0.02</td>
</tr>
<tr>
<td>3</td>
<td>1(3.8%)</td>
<td>4(6.5%)</td>
<td>2(11.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0(0.0%)</td>
<td>0(0%)</td>
<td>1(5.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1(3.8%)</td>
<td>1(1.6%)</td>
<td>0(0%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION
Discussion

The role of fungi in allergic respiratory symptoms is well established and has been described since the early 1700 (Fang et al., 2005). Although allergic diseases are increasing dramatically worldwide, only few studies evaluated asthma prevalence in Egypt, ranged from 4.8% (Khallaf et al., 1993) to 9.1 % (Mansour et al., 2014). Fungal exposure is associated with the development of asthma and rhinitis as well as epidemics of asthma exacerbations (Fukutomi et al., 2015).

In this cross sectional study, skin prick test was done for 200 patients with respiratory allergic diseases (allergic rhinitis, allergic bronchial asthma and combined allergic rhinitis and allergic asthma) with males represent 70 patients (35%) and females represent 130 patients (65%). The studied individuals included various age groups ranging from 6 years to 70 years; with mean age was 32.2 ± 14.4 years.

Out of studied patients, 40% (80 patients) had allergic bronchial asthma while 47% (94 patients) had allergic rhinitis and the remaining 13 % (26 patients) had combined allergic bronchial asthma and allergic rhinitis.

The mean age of studied patients was similar to that reported by Shakurnia et al. (2013), their studied patients were aged 4-70 years old with mean age (32 ± 14.3) and in Sathavahana et al., (2011) research, the studied age range was (37.03 ± 11.25).

Based on patients' age, the studied patients were categorized into 3 groups. The first group aged 6-14 years and included 28 patients (14%);this age range was based on International Study of Asthma and Allergies In Childhood (ISAAC) that was founded to maximize the value of epidemiological research into asthma and allergic disease. In ISAAC, the age groups of studied population was from 6 years (younger children) for reflecting early childhood years to 14 years (older children) for reflecting the period when mortality from asthma is more common and to enable the use of allergic rhinitis severity questionnaire and bronchial asthma control questionnaire. The second group aged from 15 to 44 years and included 132 patients (66%); this chosen age range was based on European Community Respiratory Health Survey (ECRHS I) that was founded to study young adults aged between 20 and 44 years of around 140,000 individuals in mainly European community to assess the prevalence of asthma and allergic disease using a standardized protocol. Finally the third age group aged 45 - 70 years and included 40 patients (20%) to reflect older adult and elderly patients.

The results of skin prick test to all studied patients revealed that 74 % (148 patients) had positive results to at least one allergen extract and only 26 % (52 patients) had negative skin prick test.
In studies conducted in many parts of the world, positive skin reaction to at least one allergen was observed in 55-97% of the patients (Donathi et al., 2011). However, in our study the percentage of positive skin prick test could be higher due to two reasons; first reason, in present study skin prick tests were used to elicit allergen extract positivity instead of intradermal tests that were used in some literatures. The second reason is related to our selected age range that was wider than these studies.

Concerning the first reason, although intradermal skin tests are more sensitive, it has many disadvantages; intradermal skin tests is less specific than SPT. Intradermal skin tests are more labor-intensive and require more precise techniques. Also these tests have occasionally been associated with serious systemic allergic reactions and even death from anaphylaxis. In the diagnosis of pollen allergy, several studies indicate that positive intradermal skin tests do not necessarily correlate with clinical symptoms (Wood, 1999) whereas there is a very good correlation between SPT results and clinical allergy symptoms (Dreborg, 1989).

Skin prick test was found to be the most reliable and available method for allergen sensitivity (Bapna, 1990). SPT results correlate with those of nasal challenge which may also be used as a surrogate to test clinically relevant sensitization (Heinzerling et al., 2013). In Pastorello et al. research on the relationship between the level of specific IgE antibody and clinical expression of allergy, it was stated that; in vitro testing had proved less sensitive, ranging from 74% to 92.2% (Pastorello et al., 1995) and European standard on skin prick test (SPTs) confirmed that, SPTs provide objective confirmation of sensitivity, whereas the relevance of such sensitivity to allergens should always be carefully interpreted in the light of the clinical history (Heinzerling et al., 2013).

On the other hand, some reports demonstrated a higher prevalence of Aspergillus sensitization in asthma with an intradermal test compared with an SPT (28.7% vs 24.8%) and even stated that: ideally, both intradermal testing and specific IgE levels should be performed for the diagnosis of fungal sensitization. However, regarding the two, the intradermal test seems superior to specific IgE levels in the diagnosis of fungal sensitization (Agarwal, 2011).

Concerning the second reason, skin test wheals increase in size from infancy to adulthood and then decline after the age of 50 (Skassa-Brociek et al., 1987); also Australian Society of Clinical Immunology and Allergy in its manual "Skin Prick Testing for the diagnosis of allergic disease" (ASCIA 2016) stated that; there are no strict age limits but skin reactions are often diminished in the very young and the elderly, making interpretation more difficult in both cases. However some studies oppose this as some authors found that, allergen responsiveness as measured by skin prick test, sex and age independently contribute to histamine sensitivity. In fact, they observed a positive correlation between the number of positive allergens at skin prick test and the wheal reaction to histamine. Also older age and male sex were associated.
with a higher response to histamine, both when studied subjects were cumulatively considered and when the evaluation was limited to allergic patients only (Bordignon et al., 2006).

In our studied patients, the results showed that 41.9% of the positive SPT patients were sensitized to at least one of the studied fungal species allergens.

Many studies were done on the prevalence of fungal sensitization in respiratory allergic diseases worldwide and in different atmospheres and ecological systems, as Shakurnia et al. (2013) in Iran showed that, 23.7% of the patients were sensitive to at least one of studied fungal species allergens. Also higher rates of sensitization to moulds are found in tropical countries like Singapore and Malaysia. Kidon et al., (2004) found in his paper; "Aeroallergen sensitization in pediatric allergic rhinitis in Singapore: is air-conditioning a factor in the tropics?" that SPT results were positive for house dust mites in 97% of children, for pets (20%), for moulds (19%), for pollens (15%), and for kapok (10%). Although Singapore is a tropical environment with a high standard of hygiene and public health care, mould sensitization was significantly more prevalent in households without air-conditioning. In studies on 85 patients with allergic rhinitis in Malaysia, prevalence of allergy to Alternaria and Aspergillus were 12.9 and 12.2%, respectively (Ishlah et al., 2005).

Also another study stated that, the prevalence of respiratory fungal allergy was found to be 20-30% of atopic individuals (Horner et al., 1995).

Nine allergology centers, in seven European countries, completed a study of prevalence of fungal sensitivity (Alternaria and Cladosporium) in respiratory allergic patients. A total of 877 subjects were enrolled in the trial; 83 (9.46%) of them turned out to be positive to at least one of the two mould allergens under investigation, Alternaria and/or Cladosporium. The age range for mould positive subjects was 5-60 years; All of these 83 (9.46%) Alternaria and/or Cladosporium sensitive subjects were sensitized to fungal allergens. Only nine (1.03%) subjects turned out to be monosensitized to Alternaria, while no subject could be classified as monosensitized to Cladosporium. The highest percentage of positive subjects was found in Spain (20%); lower percentages were found in Germany, Greece, and Austria, with the lowest percentage in Portugal (3%). Rhinitis was by far the most common diagnosis in mould-positive subjects (D'Amato et al., 1997).

This different prevalence elicited by skin prick tests can be due to number of allergen extracts used, climate changes, age of patients and humidity. Even hot and dry regions can be found its fungal sensitization like in Saudi Arabia and Kuwait. Almogren (2009) found in his literature about Airway allergy and skin reactivity to aeroallergens in Riyadh that seventy five percent of respiratory allergic patients reacted to one or more allergen extracts and a significant proportion of patients were found reacting to Moulds (18.2%) and Aspergillus fumigatus (18.2%) extracts.
Mokhtari Amirmajdi et al., (2011) conducted a case-control study that was designed by participation of 58 allergic rhinitis patients and a well-matched control group of 50 healthy volunteers. Positive results for *Alternaria* that were obtained by nasal staining and culture were seen in 34.5%, and 43.1% of cases, respectively.

One of highest prevalence of fungal sensitivity in respiratory allergic diseases was introduced by Sathavahana et al., (2011); they conducted a retrospective study on 570 patients who visited allergy clinic from January 2000 to September 2009. Skin tests were performed on 290 individuals. Based on positivity to skin tests, individuals were categorized into 3 groups according to their difference in sensitivity to antigens in different category of respiratory allergies; Positive towards Fungus, Positive towards allergens other than fungus and Positive towards other allergens and fungus. In their study, 55.3% of the individuals were sensitized to allergens other than fungus, 29.9% of individuals were sensitive to fungus only and 14.8% were sensitive to fungus plus other allergens. Therefore their study had demonstrated that overall sensitivity to fungi was 44.7%.

Still the biggest study of fungal sensitization in bronchial asthma patients is O'Driscoll et al., (2009) study. They stated that overall 66% of their patients were sensitized to one or more fungi based on SPT or specific serum Ig E or both, most of them (64%) were sensitized to more than one fungal species and the majority (76%) of those with fungal sensitization were also sensitized to non fungal allergen such as House Duse Mites, however the spectrum of patients they work on were severe asthma with medication of GINA step 4 or step 5.

Based on above mentioned studies of various prevalence results about fungal sensitivities in respiratory allergic patients, we still confirm that aerobiological studies and environmental factors play a role in that fungal prevalence variation.

When comparing the incidence of skin prick test results between various age groups, the present study showed 28 patients (14 %) had age ranges from 6 years to 14 years (young and older children); of whom, 12 patients (42.9%) showed positive results towards fungal extract. Out of 132 patients who had age of 15 years to 44 years, 40 patients (30.3%) had positive results towards fungal extracts and out of 40 patients who had age range of 45 years to 70 years, 10 patients (25%) had positive results for fungal extracts. Percentage of fungal sensitivity among patients who had aged from 6 to 14 years is higher than percentage of fungal sensitivity among other age groups.

In Galante et al., (2004) study, 139 patients aged from 6 to 55 years old was evaluated. The studied patients had respiratory allergic disease whether allergic rhinitis, allergic bronchial asthma or both. A panel of skin prick tests with moulds in addition to other inhalants allergen extracts were performed to all patients. The age group of patients from 11 to 20 years had the highest incidence of mould sensitivity
McClay et al., (2002) found that, allergic fungal sinusitis is more common among adolescent and young adults. The mean age at diagnosis was 21.9 years. In Shakurnia et al., (2013) study, the prevalence of fungal allergy in patients aged from 15 to 35 years old was 30.4% and it was higher than prevalence of fungal sensitivities among other age groups. In Sathavahana et al., (2011) study, mean age of individuals was 37.03 ±11.25 years and the most common age group for mould sensitivity was 39.25 years old.

Also O'Driscoll et al., (2009) stated that, fungal sensitization is commoner in children and declines with age. It seems likely that there is a causal relationship between mould allergy and asthma severity for some younger asthma patients.

Based on our study and above mentioned studies results about fungal prevalence among age groups, it is likely that the significance of a positive skin test to fungal allergens varies in different climatic zones but still mainly the younger patients had the higher prevalence of fungal sensitizations when tested by skin prick tests.

In spite of the importance to study fungal respiratory allergic diseases, yet limited studies in Egypt were done. In one study, a total of 56 patients with bilateral nasal polyposis were enrolled. This study was a cross-sectional descriptive study, conducted in both ENT departments and Allergy and Clinical Immunology unit of internal medicine department, Ain-Shams University from September 2009 to March 2012. The panel for skin testing was composed of histamine and saline as positive and negative control, respectively, in addition to 37 different allergen extracts for both inhaled and ingested allergens. They chose 56 patients with bilateral nasal polyposis who proved to be allergic. A total of 15 patients (26.8%) showed negative results to SPT, 41 patients (73.2%) showed positive results to SPT; of whom, 6 patients (14.6%) showed positive result to only one allergen (monosensitized), whereas the remaining patients 35 patients (85.4%) were polysensitized. The most common allergens found to be positive by SPT were: 'House Dust' in 19 patients (46.3%), followed by 'Pollens' in 18 patients (43.9%), 'House Dust Mite' in 17 patients (41.5%), and then 'Moulds' in 14 patients (34.2%) (Ashour et al., 2014).

In our work, skin prick test to fungal extracts in the studied groups showed that Alternaria alternate represented 32.2% (20 patients out of 62 patients), also Pencillium mix represented 32.2% (20 patients out of 62 patients) followed by Aspergellus mix and Chaetomium globosum each was accounting for 22.6% (14 from 62 patients) of positive fungal sensitivity patients, then in 5th order was Mucor racemosus accounting for 19.4% (12 from 62 patients). The highest prevalence of fungal sensitivities in our research is in category of mostly indoor fungi.

When correlate fungal sensitivity with predominant atopic symptoms, the present study showed that dust mites (Dermatophagoides pteronyssinus and Dermatophagoides farina) represent the highest prevalence in all patients with
positive skin prick test whether their predominant atopic symptoms were allergic rhinitis, allergic bronchial asthma or both. The study showed that *Dermatophagoides pteronyssinus* was accounting for 31.9% in patients with allergic rhinitis (30 out of 94 patients), 30% in patients with allergic bronchial asthma (24 out of 80 patients) and 38.5% in patients with combined allergic rhinitis and allergic bronchial asthma (10 out of 26 patients). *Dermatophagoides farinae* accounting for 31.9% (30 out of 94 patients with allergic rhinitis), 30% (24 out of 80 patients with allergic bronchial asthma) and 38.5% (10 out of 26 patients with combined allergic rhinitis and allergic bronchial asthma). In patients with allergic rhinitis or allergic bronchial asthma, *Alternaria alternata* and *Penicillium mix* were the second in prevalence after dust mites. In patient with combined allergic rhinitis and allergic bronchial asthma, the results showed significant sensitivities to *Pullularia pulluans*, *Merulium lacrymans* and Chenopodiaceae weed mixtures, these results were consistent with other authors' study as they found that, sensitivity to *Pullularia pulluans* is significantly associated with severe asthma (Niedoszytko et al., 2007). Also many authors studied the prevalence of house dust mites sensitization in asthmatic patients. Peat et al., (1993) found that house dust mite (*D. pteronyssinus*) sensitization was higher in severe asthmatic patients.

Ecological studies of fungal spores' prevalence found that *Aspergillus* and *Penicillium* are usually considered the major indoor fungi. *Alternaria alternate* even has been reported in house dust samples in absence of environmental mould spores. Indoor fungi are a mixture of those that have entered from outdoor and those which grows indoor (Becker et al., 1996). In a research about indoor and outdoor fungal prevalence; authors added that *chaetomium globosum* is ambient indoor air exposure, *Cladosporium herbarum* is ambient outdoor air exposure; also stated that *Alternaria alternate, Aspergillus species* and *Penicillium mix* can be outdoor and indoor exposure (Chapman, 1999).

Concerning fungal prevalence in Egypt, some authors conducted study to assess cultured airborne fungal concentrations and types in different seasons. Culturable airborne fungal concentrations were collected indoors and outdoors of 43 homes in urban and rural environments from November 2008 to October 2009 in Egypt. Fungal concentrations were significantly higher in rural environment than in urban environment with greatest dosage in the autumn and spring season. The indoor/outdoor (I/O) ratios were not statistically different between seasons. *Alternaria, Aspergillus, Cladosporium, Penicillium* and yeasts were the predominant genera indoors and outdoors and the abundance of genera varied by season and region (Awad et al., 2013). Also in their literature that was published in 2009 with studying diurnal distribution of airborne bacteria and fungi in the atmosphere of Helwan area, Egypt, the hourly concentration peaks of the bacteria and fungi appeared at 8 P.m. Fungi concentrations were significantly higher on working weekdays than weekends. *Aspergillus, Penicillium, Alternaria* and *Cladosporium* were the most predominant airborne fungal genera (Awad et al., 2009).
So the present study results about fungal species prevalence correlate with above mentioned studies about indoor and outdoor fungal concentrations in Egypt either in industrial city or rural areas. Although highest prevalence of fungal sensitivities in our research was in category of mostly indoor fungi, these fungi could also be found in outdoor environment. Moreover until standardized fungal allergen extracts are available, the exact prevalence of fungal species sensitivities will be difficult to confirm (Horner et al., 1995).

Many studies tried to answer questions of fungal species prevalence in respiratory allergic patients with skin prick tests or even sputum cultures. Authors found that most identified fungal allergens were Aspergillus, Alternaria, Cladosporium, Candida, Mucor and Pencillium in south Indian inhabitant respiratory allergic patients, mainly allergic rhinitis (Sathavahana et al.; 2011). Also other authors revealed that, higher rate of fungal culture was detected in sputum of asthma patients compared with healthy individual and Aspergillus fumigatus was the sole fungus isolated from 54% of patients, most of them (94%) was in moderate to severe disease, 27% cultured both Aspergillus fumigatus and at least one other fungus and 13% cultured one or more Pencillium species (Agbetile, 2012).

A large study with skin prick tests was applied to a cohort of 4962 respiratory subjects, aged from 3 to 80 years old. Nineteen percent of the allergic population reacted to at least one fungal extract by means of the skin test. Alternaria and Candida accounted for the largest number of positive tests, and along with Trichophyton they were the main sensitizers in the subset of patients with an isolated sensitization. Patients were divided into three subsets on the basis of skin prick tests reactivity; a single fungal sensitivity, two fungal reactions and more than two fungal extract sensitivity. The group of one fungal reaction accounted for 77.6% of subjects showing fungal sensitizations. Alternaria, Candida and Trichophyton were positive in 98% of these subjects whereas the group of two fungal sensitizations accounted for 10% of the subjects showing fungal sensitization with the same fungi that mentioned in one fungal reaction patients. The remaining subjects 12.4% were included in multiple fungal reactions. A slight increase of the prevalence of the other mould sources in multiple fungal reaction patients was recorded, but only Cladosporium reached 19% (Mari, 2003).

Also in Sharkeya, Egypt, some authors confirmed the significant relation between sputum colonization with Alternaria and bronchial asthma as well as the significant relation between colonization and sensitization to Alternaria. It indeed confirms the significant relation between sputum colonization with Alternaria and bronchial asthma ($P<0.001$) as well as the significant relation between colonization and sensitization to Alternaria ($P<0.001$). Unexpectedly, they did not find such a significant relation with Cladosporium, though being one of the most common outdoor fungi in our locality. Although they could not fully explain this finding, it
seems that the unicellular small sized conidia of *Cladosporium* may favor invasion rather than causing atopy (Azab et al., 2016).

According to local studies mentioned above and the present study, *Alternaria*, *Aspergillus* and *Penicillium* were the highest prevalence in both exposure and sensitivities to respiratory allergic patients. It was settled that increased spores counts and fungal antigen levels related to allergic symptoms.

When studying the relation of the level of asthma control and the number of positive fungal sensitivity, the results showed that 5 out of 26 patients (19.2%) of well controlled category were sensitized to only one of studied fungal extracts whereas no patients out of 18 patients of poorly controlled category were sensitized to only one of studied fungal extracts and 2 out of 18 patients (11.1%) of poorly controlled category were sensitized to 3 of studied fungal extracts. With a significant P value (0.02), it can be said that the number of fungal species extract sensitivities was positively correlated with level of asthma control.

Indeed many studies showed the relation with fungal sensitivity and severity of asthma. Some authors stated that that mould sensitization is common in patients with severe asthma requiring multiple hospital admissions. Mould sensitization was uncommon in mild asthma but very common in asthma patients with multiple admissions. Three quarters of patients with multiple hospital admissions were sensitized to moulds and half of them reacted to multiple mould allergens. The frequency of sensitization to any individual mould ranged from 26% (*Alternaria*) to 41% (*Cladosporium*) in the severe asthma group compared with 0–10% in the milder asthma groups (O'Driscoll et al, 2005).

Also a study categorized the patients according to the lifetime number of hospital admissions for asthma that reflect poor asthma control (82 patients never admitted, 53 patients had only one admission, 46 patients had multiple admissions). They found that, the number of asthma admissions was related to the number and size of positive mould skin allergy tests as 76% of patients with multiple admissions had at least one positive mould skin test compared with 16% -19% of other asthma patients. Multiple mould reactions were also much commoner in the group with multiple admissions as more than one mould sensitization was 5% in asthmatic patients without admissions and 6% with asthmatic patients with only one admission but it was significant higher reaching 50% of asthmatic patients with more than one admission (O'Driscoll et al, 2005).

Moreover O'Driscoll et al., (2009) have undertaken SPT and specific serum IgE tests to six fungi (*Aspergillus fumigatus, Candida albicans, Penicillium notatum, Cladosporium herbarum, Alternaria alternata and Botrytis cineria*) and specific serum IgE test for Trichophyton in 121 patients with severe asthma and find 29
patients (24%) were sensitized to a single fungus and seven patients (6%) were sensitized to all seven fungal species.

Also some authors found that subjects who had been admitted to the ICU with severe, potentially life-threatening asthma were significantly having one or more positive skin tests for fungal dry weather spores (Black et al., 2000). Also a cross-sectional study of 1132 adults with asthma found that sensitization to Alternaria or Cladosporium is a powerful risk factor for severe asthma in several European countries and also in Australia, New Zealand and in Portland, Oregon (Zureik et al., 2002).

From the above discussion it can be concluded that, fungal sensitization are more common in respiratory allergic disease especially with poorly controlled asthma. Also percentage of fungal sensitivity among younger group was higher than percentage of fungal sensitivity among other age groups. Alternaria alternate and Pencillium mix represented the highest prevalence among positive fungal sensitivity patients
SUMMARY AND CONCLUSIONS
Summary and conclusion

Allergies are increasing dramatically worldwide. Approximately 10% to 30% of the world’s adult population and up to 40% of children are affected by some form of allergy. Few studies evaluated asthma prevalence in Egypt, Ranged from 4.8 % to 9.1%.

There is a strong epidemiologic association between rhinitis and asthma when both relate to inhalant allergy, a finding that represent the pathophysiologic link between these two disorders so the concept ‘‘one airway, one disease’’ has been labeled accordingly.

Aeroallergens are relatively large and complex particles, such as pollens, moulds, insect parts, animal dander, plant fragments, and house dust mites that are capable of eliciting allergic reactions in susceptible persons. Fungi are eukaryotic, unicellular or multicellular. They are mostly spore-bearing organisms that exist as saprophytes or parasites of animals and plants. The fungi constitute a very large and diverse set of organisms, and the taxonomy is complicated. Classification schemes have undergone numerous revisions to develop a system easier to follow. Most fungi produce sexual and asexual spores and the taxonomy is based on that spore characteristics.

The role of fungi in producing allergic and respiratory symptoms is well established and has been described since the early 1700s. Of these aeroallergens Indoor moulds are most problematic in homes with high humidity, standing water, or water damage and in outdoor high dose exposures. Aspergillus, Cladosporium, Alternaria, Curvularia and Fusarium species have been reported as the most prevalent fungi of indoor and outdoor fungi around the world.

The major taxonomic groups of fungi that are currently recognized are the ascomycetes, basidiomycetes, and zygomycetes. Some authors also refer to a separate group, the deuteromycete that contains a large number of allergenic fungi that reproduce asexually by the differentiation of specialized hyphae called conidiophores. Among these, about 80 mould genera have been shown to induce type I allergies in atopic individuals. The most important allergenic fungi belong to the genera Alternaria, Aspergillus and Cladosporium, whereas members of the genera Candida, Penicillium, Clavularia and others seem to be less important as allergenic sources.

Fungi can cause problems to the lung either by acting as aeroallergens or by acting as a pathogen causing infection or even both mechanisms. Fungal allergic burden to human is broad and still need much clarification about diagnostic criteria to diagnose each such as Allergic bronchopulmonary aspergillosis (ABPA) , severe asthma with fungal sensitization (SAFS) and Allergic fungal sinusitis (AFS)
Many studies in past decades showed that severity of respiratory allergic disease is correlated with fungal sensitivities. These studies began early in the 1870s by Charles Blackley with inhaling fungi from straw bristle mould Chaetomium elatum and Penicillium glaucum. Many studies later stated that sensitization to Alternaria or Cladosporium is a powerful risk factor for severe asthma in several European countries. Asthma deaths, multiple hospital admissions, respiratory symptoms and peak expiratory flow rates can be adversely affected by high fungal spore dose in outdoor air. Although it is reasonable to expect that exposure to fungi in sensitized individuals with asthma is likely to lead to symptoms, it is less clear that exposure to fungi is likely to increase the risk of developing asthma.

The aim of present study is to evaluate the prevalence of sensitization to various species of mould and yeast allergens among other common aeroallergens in Egyptian patients with respiratory allergy.

The present study included 200 patients diagnosed with bronchial asthma and/or allergic rhinitis. For each patient, the following had been done: Detailed allergic history and clinical examination with assessment of asthma control level, allergic rhinitis severity, Spirometry and SPT to aeroallergens using commercial standardized extracts. Extracts for skin testing included standardized extracts of 10 fungal allergen extract in addition to other 6 aeroallergens, positive and negative controls.

Mean age of the patients was 32.2 ± 14.4 years with a range from 6 -70 years old. Females represented 65% of the study population while males represented 35 %. Of studied patients, 14 % were aged between 6-14 years; 66 % had age range from 15-44 years and 20% were aged between 45 and 70 years. Asthma represented 40% of the study population while allergic rhinitis represented 47% and combined allergic bronchial asthma with allergic rhinitis represented 13 %.

The results of skin prick test to all studied patients revealed that 74 % had positive results to at least one allergen extract and only 26 % had negative skin prick test. As regards patients with positive skin prick test to at least one allergen extract, positivity towards fungus extract was 41.9 % and positivity towards other allergen was 58.1 %.

Regarding the severity degree of allergic rhinitis, there were 28.3 % of studied allergic rhinitis patients had mild degree and 71.7 % patients had moderate to severe allergic rhinitis. Regarding the control state of bronchial asthma, there were 26 out of 106 patients (24.5 %) were well controlled, whereas, 62 out of 106 patients (58.5 %) were partially controlled and 18 out of 106 patients (17 %) were poorly controlled.

When comparing the incidence of skin prick test results between various age groups, the study showed 14 % of 6 years to 14 years; showed positive results towards fungal extract in 42.9%. Out of 132 patients who had age of 15 to 44 years, 30.3% of them had positive results towards fungal extracts and out of 40 patients who
had age range of 45 to 70 years, 25% of them had positive results for fungal extracts. Percentage of fungal sensitivity among age group who had aged from 6 years to 14 years was higher than percentage of fungal sensitivity among other age groups.

Regarding results of skin prick test to common fungal extracts in the studied positive fungal population, *Alternaria alternate* represents 32.2%, *Pencillium mix* represents 32.2% followed by *Aspergillus mix* and *Chaetomium globosum* each accounting for 22.6% of positive fungal sensitivity patients, then in 5th order was *Mucor racemosus* accounting for 19.4%. Regarding the results of skin prick test to common non fungal allergen extracts in the studied positive skin prick test study population, the study showed that dust mites extracts with its two species *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* sensitivity represent the highest prevalence of allergen extracts sensitivity. In patients with combined allergic rhinitis and allergic bronchial asthma, the results showed significant sensitivities to *Pullularia pulluans*, *Merulium lacrymans* and *Chenopodiaceae* weed mixtures.

Studies of fungal ecological systems found that *Aspergillus* and *Pencillium* are usually considered the major indoor fungi. *Alternaria alternate* has been reported in house dust samples in absence of environmental mould spores and so it can represent outdoor and indoor mould exposure. Egyptian ecological studies about fungal exposures found that, fungi concentrations were significantly higher on working weekdays than weekends. Although highest prevalence of fungal sensitivities among respiratory allergic patients in our research was in category of mostly indoor fungi, these fungi could also be found in outdoor environment.

Many studies tried to answer questions of fungal species prevalence in respiratory allergic patients with skin prick tests or even sputum cultures. These studies denoted that *Aspergillus*, *Alternaria*, *Cladosporium* and *Pencillium* were the most identified fungal allergen.

When studying the relation of the level of asthma control and the number of positive fungal sensitivity, The results showed that highest percentage (19.2%) of patients in well controlled category were sensitized to only one of studied fungal extracts, whereas, the highest percentage (11.1%) of patients in poorly controlled category were sensitized to 3 of studied fungal extracts. With a significant P value 0.02, it can be said that the number of fungal species extract sensitivities was related with level of asthma control.
Conclusions

To conclude, skin prick tests with studied aeroallergen extracts were positive to at least one of studied extracts in majority of studied patients. Up to half of positive studied patients were positive to at least one of the studied fungal allergens. Percentage of fungal sensitivity among age group who had aged from 6 to 14 years old i.e. younger group, was higher than percentage of fungal sensitivity among other age groups.

*Alternaria alternate* and *Pencillium mix* represented the highest prevalence among positive fungal sensitivity patients followed by *Aspergellus mix* and *Chaetomium globosum*, then in 5th order was *Mucor racemosus*.

In patient with combined allergic rhinitis and allergic bronchial asthma, the results showed significant sensitivities to *Pullularia pulluans*, *Merulium lacrymans* and *Chenopodiaceae weed mixtures*.

Percentage of the patients that were sensitized to only one of studied fungal extracts in well controlled asthmatic patient's category was higher than percentage in poorly controlled patient's category. In poorly controlled asthmatic patient's category, there was increase in the percentage of patients that were sensitized to 3 of studied fungal extracts. It can be said that the number of fungal species extract sensitivities were related with level of asthma control.
RECOMMENDATIONS
**Recommendations**

We recommend that subjects with severe asthma and positive fungal SPT should be warned about weather conditions that increases spores count to avoid fungal exposure as prevention control measures with proper education to avoid mould contact and desensitization to will pave the road to more easily control of severe respiratory allergic patients sensitized to fungal allergens.

Aerobiological studies are needed in Egypt to quantify more closely dose response relation of fungal exposure and asthma and to develop more effective environmental and therapeutic interventions.

Studies should be done to evaluate the efficacy of fungal extract immunotherapy in Egyptian respiratory allergic patients. Also it is highly recommended that when giving immunotherapy to patient with respiratory allergic disease with fungal sensitivity and other allergens sensitivity, it should not be mixed in same immunotherapy vial, as it contains proteases enzymes which degrade other allergens mixed in same immunotherapy (IT) solution.
REFERENCES


Adkinson NF, Bochner BS, Busse WW, Holgate ST, Lemanske RF, Simons FER. *Middleton’s Allergy: Principles and Practice*. 7th. PA: Mosby Elsevier; 2009.


Akurst L, Borch SM. Partial purification and characterization of two Cladosporiumherbarum allergens. *Int Arch Allergy Appl Immunol* 1979; 60:68–79.


Aubier M, Neukirch C, Peiffer C, Melac M. Effect of cetirizine on bronchial hyperresponsiveness in patients with seasonal allergic rhinitis and asthma. *Allergy* 2001; 56:35-42.


Banerjee B, Kurup VP, Greenberger PA. Cloning and expression of *Aspergillus fumigatus* allergen *Asp f 16* mediating both humoral and cell-mediated immunity in allergic bronchopulmonary aspergillosis (ABPA). *Clin Exp Allergy* 2001; 31:761–70.


Bennich HH, IshizakaK, JohanssonSG, RoweDS, StanworthDR, TerryWD. Immunoglobulin E, a New Class of Human Immunoglobulin.*the Journal of Immunology*1968; 100:1143


Blackley CH. Experimental researches on the causes and nature of catarrhus aestivus (hay-fever or hay-asthma). Baillière, Tindall & Cox. 1873.


116


Boulet LP, Turcotte L, Laprise C, Lavertu C, Bedard PM, Lavoie et al. Comparative degree and type of sensitization to common indoor and outdoor allergens in subjects with allergic rhinitis and/or asthma. *Clinical & Experimental Allergy* 1997; 27: 52-59.


Chauhan BF, Ducharme FM. Anti-leukotriene agents compared to inhaled corticosteroids in the management of recurrent and/or chronic asthma in adults and children. *Cochrane Database Syst Rev.* 2012.


Downs SH, Marks GB, Mitakakis TZ. Having lived on a farm and protection against allergic diseases in Australia. *ClinExpAllergy* 2001; 31: 570-5.


Durham SR, Smurth WL. Local IgE synthesis in allergic rhinitis and asthma. *Current allergy and asthma reports*, 2002; 2: 231-38.


Franks TJ, Galvin JR. Hypersensitivity pneumonitis: essential radiologic and pathologic findings. *Surgical Pathology Clinics* 2010;3(1), 187–98,


Galante D, Tassinari PA, Conesa A, Trejo E, Bianco NE. Specific IgE to indoor molds in patients with respiratory allergies. *Journal of Allergy and Clinical Immunology* 2004; 113(2): S144.


Holgate ST. Innate and adaptive immuneresponses in asthma. *Nat Med* 2012; 18:673–83


Kauffman HF. Immunopathogenesis of allergic bronchopulmonary aspergillosis and airway remodeling. Front Biosci 2003; 8: E190-E196.


Loza MJ, Chang BL. Association between Q551R IL4R genetic variants and atopic asthma risk demonstrated by meta-analysis. *Journal of Allergy and Clinical Immunology* 2007;120: 578-85.


Maansi Vermani1, Vannan Kandi Vijayan, Mahendra Kumar Agarwal. Identification of *Aspergillus* (*A* *flavus* and *A* *niger*) Allergens and Heterogeneity of Allergic Patients’ IgE Response. *Iranian Journal of Allergy, Asthma and Immunology* 2015; 14(4):361-69.


Mitakakis TZ, Barnes C, Tovey ER. Spore germination increases allergen release from Alternaria. *J Allergy Clin Immunol* 2001;107:388–90


Murdoch JR, Lloyd CM. Chronic inflammation and asthma. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 2010;690: 24-39.


Niedoszytko M, Chełmińska M, Jassem E, Czestochowska E. Association between sensitization to Aureobasidium pullulans (Pullularia sp) and severity of asthma. *Annals of Allergy, Asthma & Immunology* 2007; 98,153-56.


O'Driscoll BR, Hopkinson LC, Denning DW. Mold sensitization is common amongst patients with severe asthma requiring multiple hospital admissions. *BMC Pulmonary Medicine* 2005; 5:4


Refaat M, Mansour T, Talaat S, Ahmed Z, Eman O, Eman E et al. Role Of House Dust Mites In Allergic and Non Allergic Nasal Diseases *the journal of allergy and clinical immunology* 2014 ; 133, 2, AB46


Shen HD, Tam MF, Chou H, Han SH. The importance of serineproteinases as aeroallergens associated with asthma. *Int Arch Allergy Immunol* 1999;119:259–64.


Skassa-Brociek W. Skin tests reactivity to histamine from infancy to elderly. *J Allergy Clin Immunol* 1987; 80:711


Taramarcaz P, Gibson PG. Intranasal corticosteroids for asthma control in people with coexisting asthma and rhinitis. *The Cochrane Library.* 2003


Warrington R, Watson W, Kim HL, Antonetti FR. An introduction to immunology and immunopathology. *Allergy, Asthma & Clinical Immunology* 2011 ;7:S1


Ying S, Meng Q, Zeibecoglou K, Robinson D S, Macfarlane A. Eosinophil chemotactic chemokines (eotaxin, eotaxin-2, RANTES, monocyte chemoattractant protein-3 (MCP-3), and MCP-4), and CC chemokine receptor 3 expression in bronchial biopsies from atopic and nonatopic (Intrinsic) asthmatics. *The Journal of Immunology*; 1999; 163: 6321-29.


الملخص العربي

إن امراض الحساسية تزداد عالمياً فطرياً للابحاث الانتشار وجد أن حساسية الصدر تتراوح ما بين 4.8% و 9.1% بين المصريين ووجد أن امراض الحساسية تبلغ 30% عند البالغين وحتى 40% عند الأطفال.

من مسببات الحساسية التنفسية كثيرة فمنها حبوب اللقاح والجراثيم،平方米 الفطريات وحشوات الفطريات، وحبوب اللقاح.

أن الفطريات قد تكون حيوية خلية أو متعددة الخلايا لكن لا تحتوي على الكولويفيل وهم في أغليتتها حاملاً للإمراض وهاك من الفطريات ما يسمى الطور الرماد الذي تعيش على الأجسام المبتهجات أو تعايشات كالطلقيات على الحيوانات والنباتات والعفن هو نوع من الفطريات التي تتنج وحدات إنجابية مجهري ومتالي كلهما الفطريات والعفن ليس متصرفين تماماً، ولكن غالباً ما نستخدمن بالتبادل كأن نصف شيئاً أو شيئاً واحداً.

والتاريخية الفرعية للاتحاد الرئيسي للجمعيات المناعية على مستوى العالم (IUIS) استقرت على ما يسمى تصنيف مستويات الحساسية الفطرية، وتم إدراج الكنز من الأنواع الفطرية التي تنتمي إلى عائلة اسكوميكوتا وبايديوميكوتا.

إن دور الفطريات في أمراض الحساسية قد بدأ البحث فيه منذ عام 1700 ميلادي و قد وجد أنه مما لا يمكن عن 600 نوع من الفطريات على اتصال مع البشر، ولكن أقل من 80 نوع فقط هو ما تم التعرف عليه ووصفه في الدراسات الوبائية على البكتيريا المغلقة.

إن المستعمات من الفطريات مثل اسبريليس فاميجيتا و الترناريا الترناريا و كلاودسبرومير هيبليرم و فطرية البنسيليم و الفيوزاريوم كل ذلك متواجد حولنا في بيئتنا، وبالتالي، ليس هناك بيئة خالية من الفطريات.

أن التحسن الفطرية هو عامل مهم في المرضا الذين يعانون من أمراض حساسية الجهاز التناسلي، ويتطلب دوراً رئيسي في تطوير واستمرار وشدة المرض بمجرد الهواء السفلي للمرضين ومن أهم أمراض الحساسية التي تسببها الفطريات هي حساسية الرئة، حساسية الأنف، التهاب الجيوب الأنفية، داء فطري للجهاز القصبي، التهاب والتهاب رئوي فطري حساسية.

في هذه الدراسة قد تم بحث 200 مريض مصابين بحساسية الجهاز التناسلي ومنهم من هو مصاب بحساسية الصدر ومنهم ما هو مصاب بحساسية الأنف وباقي مصابين بكتلة نوع حساسية المصدية. وقد تم عمل الاتي لكل المرضى الخاضعين للدراسة: اخذ التاريخ المرضي، واختبار التحسس من الفحص الالكتروني مع معرفة دجاج التحكم في حساسية الصدر ومعروفة شدة حساسية الأنف. كما تم عمل العديد من الفحوصات وهي: صورة دم كاملة ووظائف تنفسي، اختبار التحسس بالوخ الأذى باستخدام مستخلصات قياسية موحدة من 10 أنواع من الفطريات بالإضافة إلى 6 من المثيرات الهوائية الأخرى.
كانت اعمار المرضى تتراوح ما بين 6 الى 70 عام و كانت نسبة السيدات أكبر و تمثل 65 في المائة و كانت اغلبية المرضى من 15 حتى 44 عام.

وقد أدى نتائج الدراسات ان حوالي 74 في المائة من المرضى ايجابيون لاختبار الوخز بالابر و منهم 41.5 في المائة ايجابيين لاحدائ مستخلصات الفطريات و حوالي 42.9 في المائة من الذين تتراوح اعمارهم من 6-14 عام كانت نتائجه ايجابية لاحدائ مستخلصات الفطريات في حين ان النسبة كانت اقل في من تتراوح اعمارهم من 15-44 عام و أيضاً الذين تتراوح اعمارهم من 45-70 عام.

أظهرت الدراسة ان فطريات الترنابيا انترنا و البنسيلم يمثلان المرتبة الأولى في البحوث لدى العينات المدوينة من المرضى ذوي الحساسية الصدرية و تبلغ نسبة كل منها 32.2 في المائة من مستخلصات الفطريات العشرة التي تمت دراستها وياتي في المرتبة الثانية فطر إسبرجيلس و بلغت نسبة 22.6 في المائة وهذه الفطريات تتواجد كثيراً داخل البياني على الرغم من وجودها أيضاً في الهواء الطلق.

عند دراسة نسبة التحكم في الحساسية الصدرية و علاقتها بالتحسس الفطرية وجد أنه كلما زاد عدد الفطريات الإيجابية عند عمل اختبار الوخز بالابر فإن درجة التحكم بحساسية الصدر تكون ضعيفة.

لا توجد الكثير من القياسات لدرجة انتشار التحسس من الفطريات بين المرضى المصريين الذين يعانون من حساسية الجهاز التنفسي باستخدام اختبار الجلد و يفسر ذلك نظراً لعدة عوامل منها استخدام مقتطفات الحساسية غير القائمة لاختبار الجلد، و عدم وجود خرائط بيئية مكتملة لبؤس العفن أو معرفة اعدادها و استخدام خليط لاختبار الجلد من مختلف أنواع الفطريات بدلاً من كل على حدة و لهذا كان هذا البحث لمعرفة نسبة انتشار التحسس من الفطريات بين مرضى حساسية الجهاز التنفسي.

وفي النهاية يتضح ان التحسس للفطريات موجود بنسبة كبيرة لدى المرضى المصريين المصابين بحساسية الجهاز التنفسي خاصة مرضى الحساسية الصدرية ذوى درجة التحكم ضعيفة و من هنا نؤكد نؤكد ان التحسس للفطريات يلعب دوراً رئيسي في تطور و استمرار و شدّة المرض بمجرى الهواء السفلي للرئتين بل قد تكون عامل مسبب لحساسية الصدر في الأطفال منذ الصغر.
مدى انتشار التحسس للعفن والخميرة المسببة للحساسية لدى المرضى المصريين الذين يعانون من حساسية الجهاز التنفسي

رسالة بحثية

مقدمة توطئة للحصول على درجة الدكتوراه في الأمراض الباطنية

باستخدام

مدحت ماهر عبد اللطيف العماوي

ماجستير أمراض الباطنة
كلية الطب - جامعة بنها

تحت إشراف

ا.د. عاطف أحمد إبراهيم

استاذ الباطنة
كلية الطب
جامعة بنها

ا.د. نبيل السيد خطاب

استاذ الباطنة
كلية الطب
جامعة بنها

ا.د. محمد نظمى فارس

استاذ الباطنة والحساسية
كلية الطب
جامعة عين شمس

ا.د. ماجد محمد رفعت

استاذ الباطنة والحساسية
كلية الطب
جامعة عين شمس

كلية الطب - جامعة بنها
2017