The Role Of *Candida albicans* And *Escherichia coli* Lipopolysaccharide Allergens As A Causative Agents Of Type I Hypersensitivity

A thesis Submitted to the
College of Medicine – University of Basrah
In Partial Fulfillment of the Requirements for the
Degree of Master of Science
(Medical Microbiology)

By

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August, 2005

بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

نَرۡفَعُ دَرَجَٰتٖ مَّن نَّشَآءُۗ

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صَدَقَ اللهُ العلي العَظيمُ

سورة يوسف، الآية 76

الإهـــداء

إلى

مثلي الأعلى ومعلمي الأول

وصاحب الفضل الأكبر

والدي ... رحمه الله تعالى

إلى

من أوتني عطفها ورعتني بحنانها

ووسدتني راحتها .. نور عيني

أمي ...حفظها الله ورعاها

إلى

مصـدر طمـوحـي ونجـاحي

زوجتي الفاضلة ... عبير

وأطفالي الأحباء

أهدي جهدي المتواضع

ثمرة عطائكم

وميض

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Acknowledgments

Firstly, I wish to express my sincere gratitude for God who made me finish my work during difficult times.

I wish to express my gratitude and deep appreciation to my supervisors Dr.Sundus S.Bakr and Dr. Hadi A.Al-Fyadh for their helps and advice throughout the course of the research.

I would like to thank the dean of college of Medicine, the head of microbiology department, and all members of microbiology department of Basrah Medical College for their kind help and continuous support.

I am also deeply and extremely grateful to laboratory staff of the Asthma and Allergic Disease Center particularly Mr. F. Hmod and Miss R. Waleed.

I'd like to express my deep thanks to staff of department of microbiology, college of veterinary medicine particularly Dr. Fawziah A.Abdulla, Head of the department of microbiology and Dr. R. Munthor.

Finally , I'm extremely grateful to my family for their support during the period of study.

List of Abbreviations	
Abbreviated Form	**Full Form**
Ab	Antibody
Ag	Antigen
C. albicans	*Candida albicans*
CMI	Cell mediated immunity
DTH	Delayed type hypersensitivity
E. coli	*Escherichia coli*
ELISA	Enzyme linked immunosorbent assay
Fab	Fragment antigen binding
Fc	Fragment crystallizable
GM-CSF	Granuilocyte monocyte–colony stimulating factor
GYEP	Glycoset yeast extract pepton media
IFN- γ	Interferon - γ
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
IU/ml	International unit per mill
KD	Kilodalton
LPS	Lipopolysaccharide
MW	Molecular weight
OD	Optical density
PAF	Platelets activating factor
RAST	Radio allergosorbent test
Th	T – helper cell
μm	Micrometer

SUMMARY

An Allergic extracts from *Candida albicans* were prepared by extraction, followed by purification and fractionation by gel filtration, three major peaks were obtained with a molecular weight 37KD, 43KD and 46 KD. Also, Lipopolysaccharide (LPS) was extracted and purified from cell wall of *Escherichia coli* and used as allergen.

Total IgE ELISA tests was performed on one hundred fifty patient sera samples, who attending the Center of Asthma and Allergic diseases in Basrah, and one hundred healthy individuals as control groups, patient's age ranged between (10-60) years. One hundred thirty nine (139) patients had positive total IgE ELISA results.

These 139 patients then tested with locally prepared potential *C. albicans* allergen peak (I, II and III), standard *C. albicans* allergen and locally prepared *E. coli lipopolysaccharide* allergen.

There were significant difference among age groups regarding the number of patients who had specific IgE positive results for both allergen extracts, the highest rates were observed in male and female of 10-30 years of age.

In addition, a significant difference, regarding the rate of positive responses, between males and females examined with these allergens, the highest rate of positive IgE ELISA results was observed in females in comparison with males.

In this study the rate of patients who suffering from asthma and had positive IgE ELISA result for LPS allergen was highest than those who suffering from other atopic diseases, while pruritis was more prevalent in patient tested for *C.albicans* allergy.

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Chapter One

Introduction

INTRODUCTION

Adaptive immunity serves the important function of host defense against microbial infection. In particular, it is able to provide rapid protection against a repeated challenge with the same or similar foreign organisms or toxin, but immune responses are also capable of causing tissue injury and disease. Disorder caused by immune responses are called hypersensitivity diseases. Hypersensitivity may occur as an exaggerated form of an appropriate response and damaging to the host [(1)].

In the early 1960s, hypersensitivity reactions were divided into four types, designated I –IV by Coombs and Gell[(2)]. Type I, IgE mediated reactions are stimulated by the binding of IgE (via its Fc region) to high – affinity IgE – specific Fc receptors ($Fc_{\varepsilon}RI$) expressed on mast cells and basophils. When cross linked by antigens, the IgE antibodies trigger the mast cells and basophils to release pharmacologically active agents that lead to clinical manifestations including rhinits, astham and in serve cases, anaphylaxis. Reaction are rapid, occuring within minutes after challenge, that is reexposure to antigen[(3)]. Type II, the antibody is directed against antigen on an individual's own cells (target cell) or foreign antigen, such as transfused blood cells. Type III, immune complexes reactions occur when complex of antigen and IgM ot IgG antibody accumulate in the circulation or

in tissue and activate the complement cascade. Type IV, cell mediated immunity (CMI) reactions, also called delayed type hypersensitivity (DTH) is mediated by T-cell associated effector mechanisms rather than antibody[4].

Most allergic IgE responses occurred on mucous membrane surface in response to allergens that enter the body by either inhalation or ingestion[5].

Endotoxins are released from the membrane of Gram-negative bacteria present in the environment and in oral and nasal cavities. They are proinflammatory substances that could participate in bronchial obstruction and hyperreactivity in asthmatic patients [6]. Detection of IgE anti-lipopolysaccharide may indicate that allergic mechanisms are involved in the response to endotoxin[7].

The yeast *Candida albicans* is a common commensal of the human oral and vaginal mucosae and gastrointestinal tract, and causes to only opportunistic infections in immunocompromised patients but also allergic reactions in people sensitized to *C. albicans.*

A high IgE titer is thought to be a risk factor for type I hypersensitivity in human[8]. The IgE titer was measured in a radio allergosorbent (RAST) or enzyme – linked immunosorbent assay (ELISA). Because standard antigens have not been established, only overall titers against mixed antigens including

protein and polysaccharides were obtained. However, it has been shown that antigenic components of *C. albicans* can stimulate immediate hypersensitivity responses in animals [9].

The aqueous extracts used in testing for IgE antibodies in allergic patients may contain a number of allergens in addition to non allergenic components so the purification and characterization of these allergenic components become very important for the development of specific reagents for diagnosis and treatment as immunotherapy. Purified allergen are also, essential reagents for research studies on structure – function relationship and for genetic studies because the purified extracts of allergen contain non – relevant materials that may disturb diagnostic tests causing unreliable results [10].

The aims of the study:

1. To prepare and characterize *Candida albicans* and *Escherichia coli* lipopolysaccharide allergens.

2. To measure total and specific IgE values against *C. albicans* and *E.coli* lipopolysaccharide allergens in patient's sera.

3. To detect types of allergic disorders associated with each prepared allergens.

4. To compare the locally prepared *C. albicans* allergens with the standard *C. albicans* allergen.

Chapter Two

Literature review

Chapter Two
Literature Review

2.1. Atopic diseases

The term atopy refers to clinical conditions caused by an inappropriate immune responses to innocuous proteins in genetically predisposed persons[11].

Atopic individual are exposed to several types of organic materials, e.g. mites, animal derived materials, feeds, bacteria ad fungi. Continuous exposure may lead to sensitization and to the development of allergic symptoms[12].

It becomes clear that, injection of any protein into an individual with immediate hypersensitivity to that protein can induce anaphylaxis, thus, anaphylaxis occurs when a patient with immediate hypersensitivity is exposed to a relevant allergen in such away that antigen enter the circulation rapidly. This can occur after injection of penicillin and eating an allergen such as peanut or shellfish[13].

The immune system serves as the body's defence mechanism against the countless different substances present in the air that we breath or food we eat and things we touch, within this immense group of materials, the term allergen refers to any substance that can

trigger an allergic response represented by production of antibodies circulating in the blood stream and present in almost all body fluids[14].

The earliest description of any human ailment resembling hay fever was noted in Persia around 865 AD by Mohammed Al-Razi. While the earliest report in western literature that may describe allergic rhinitis did not appear until the 16th century when Leonhardus Botallus of Pavia described symptoms resembling an allergic reaction to roses[15]. After Botallu's brief description, no clear reference to any condition resembling allergic rhinitis appeared until the 1800s. In 19th century Dr. John Bostock described his own disorder of seasonal illness involving watery eyes, sneezing and runny nose in England. His description in 1819 is considered to be the firsts clear case description of atopic disorder. Since the time of Bostock, the prevalence of allergic rhinitis has been increasing in UK[16]. Asthma prevalence rates also increased from 8.5 per 1000 in 1955 to 17.8 in 1981[17]. The increase in the prevalence of atopic disease has not been limited to England. Instead, the trend seems to be global, or at least, common to the industrialized countries where it has been studied[16,17,18,19].

2.2. Etiology of the allergy

The etiology of atopy is unknown, there is substantial evidence for a complex of genes with variable degree of expression encoding protein factors, some of which are pathogenic and other protective. These genes and genes clusters occupy positions on at least eleven different chromosomes in the human genome[20]. These are the ones that in fluency the propensity for atopy through the regulation of total IgE production and specific IgE antibodies to allergen epitopes, cytokines and their receptors, enzyme and receptors for mast cell mediators, and undoubtedly other factors involved in disease pathogenesis[21].

The abnormal IgE response of atopic individuals is at least part genetic, it often runs in families. Atopic individuals have abnormally high levels of circulating IgE and also more than normal numbers of circulating eosinophils. These individuals are more susceptible to allergies such as hay fever, eczema, and asthma. The genetic propensity to atopic responses has been mapped to several candidate loci. One locus, on chromosome 5q, is linked to a region that encodes a variety of cytokines, including interleukin -3 (IL-3), IL-4, IL-5, IL-9, IL-13 and granulocyte monocytes-colony stimulating factor (GM-CSF)[22].

A second locus, on chromosome 11q, is linked to a region that encodes the β-chain of the high-affinity IgE receptor [23].

It is known that inherited atopy is multigenic and that other loci probably involved. In fact, as the sequencing of the human genome progresses, these other candidate genes may be revealed[24].

Environmental factors also play a key role in the pathogenesis of atopic asthma[25]. And it is likely that asthma reflects exposure to environmental trigger factors in genetically susceptible individuals. Three primary environmental exposures that lead to airway inflammation and allergic symptoms have been identified : exposure to common aeroallergens such as house dust mites, environmental tobacco smoke exposure and exposure to viral respiratory infections.

In susceptible individuals, this exposure may result in disease from early childhood. The strong association with environmental factors that are themselves clustered within families complicates gene discovery in asthma [26,27,28].

2.3. Pathogenesis and Clinical manifestation of atopic allergy

Atopic allergy is defined as a hereditary tendency to develop IgE antibodies to common substances in the environment[29].

When these substances (allergens) come into contact with the immune system, they are caught by IgE molecules located on the

surface of mast cells and basophiles via high-affinity receptors[30]. Mast cells are found in connective tissues particularly around veinules and in mucosal membranes, although derived from the same progenitor cell under the influence of IL-3[31].

If these IgE antibodies are cross-linked by an antigen, a signal is being transmitted from the receptors that lead to an increase in cytoplasm free calcium. This lead to cell degranulation and to the release of several inflammatory mediators such as histamine, heparine, eosinophil and neutrophil chemotactic factors.

In addition, activation of mast cells, formation of leukotriens, prostaglandins and platelets activating factor (PAF). These mediators normally assist the immune defence against microbial infections by enhancing the acute inflammatory reaction. When a sensitized person is exposed to allergic material, the mediators release are excessive and allergic symptoms develop[32].

There are different types of allergic disease, which include allergic rhinitis, allergic conjunctivitis, allergic dermatitis, urticaria or hives and asthma[30]. Symptoms of allergic rhinitis, commonly referred to as "a hay fever" occur when allergens touch the lining of the nasal mucosa of a person who is sensitive to that particular allergen. Allergic rhinitis is characterized by itchy, runny nose if the person continues to have daily, on going exposure to the responsible

allergens[33]. Similar to allergic rhinitis allergic conjunctivitis occurs when the eyes react to allergens with symptoms of reddening, itching and swelling.

Symptoms of atopic dermatitis also called eczema, often result from exposure to allergen. Dermatitis symptoms include itching, reddening, and flaking or peeling of the skin[34,35].

In urticaria or hives, another allergic skin reaction is characterized by itchy red bumps that can occur in clumps and be either large or small[36]. Allergic asthma is characterized by bronchial hyperreactivity to various stimuli followed by a secondary increase of inflammatory cells in the airways epithelium and in bronchial secretions[37].

The inflammatory characteristics of asthma and respiratory infections are different. Two subsets of T-helper (T_h) lymphocytes have been defend and are distinguishable by a differing pattern of cytokine release. T_{h1}-type cells stimulate the recruitment and activity of macrophages and/or immunity activated by microbial antigens, typically resulting in IgG antibody production without an increase in IgE[38].They produce IL-2, interferon-γ (IFN-γ) and tumor necrosis factors.

T_{h2} cells responses are important in the allergic response and asthma, producing the cytokines IL-4, IL-5, IL-6, IL-10 and IL-13 that are involved in humeral immune responses of elevated IgE levels and eosinophilia[38].

2.4. Allergens

2.4.1. General properties

Allergens are widely distributed in nature. They are usually proteins or glycoproteins originating from plants, microbes, vertebrates and invertebrates[39]. Allergens are classified as major, intermediate and minor. A major allergen is defined, as one that binds IgE antibodies in 50% of sera from patient's allergic to the substance. It is usually acidic globular molecules that are stable to enzymatic digestion or chemical denaturation. Their size is usually between 10000-40000 Daltons[40]. A minor allergen binds IgE antibodies in not more than 10% of the sera from the same patient's population, allergens with binding capacities between these two levels are referred to as intermediate allergen[41].

The primary characterization of allergens relates to their route of exposure, this includes inhaled allergens, food, drug allergens and antigens from fungi growing in the body and venoms[42]. The routes are important because they define the way in which the antigens are presented to immune system[43].

2.4.2. Lipopolysaccharide (LPS)

LPS is a component of the cell membrane of Gram-negative bacteria and is responsible for initiating the inflammatory events of Gram negative sepsis[44].

Bacterial LPS (the major constituent of endotoxines) are Gram-negative bacterial constituents present in the oral and nasal cavities and also, present in the environment. Endotoxines are found in the commercial milk samples (30-130µg/ml)[45], that could reach the lung by gastroesophageal reflux or by inhalation. Airborne endotoxines can also be detected in domestic water (0.04-1µg/ml)[46], that could be inhaled in bath rooms and in air humidification systems (0.13-0.39 µg/m^3)[47].

Lipopolysaccharide (LPS) binds to LPS binding protein, producing stimulation of macrophages and subsequent release of inflammatory mediators such as tumor necrosis factor-alpha; interleukin-6). This activation of inflammatory mediators initiates the physiologic changes characteristic of the sepsis syndrome with continued activation leading to septic shock and multiple organ failure[48].

There are a number of mechanisms in immunocompetent hosts which modulate the response to LPS. The endogenous intrerleukin-1 receptor antagonist directly antagonizes the interleukin-1

proinflammatory effect. Potentially, binding of LPS to LPS-binding protein can be controlled through down regulation of LPS-binding protein availability. Also, expression of LPS receptor on macrophages (CD14) could be down regulated[49].

Lipopolysaccharide have the capacity to interact with the cellular mediator system of the host, including platelets, mast cells, basophiles, neutrophils, monocytes, macrophages and endothelial cells[50]. Furthermore, LPS may activate major humoral mediation system such as complement and the coagulation systems (including the kinin system). These components of the cellular and humoral responses are present and active in bronchial and pulmonary tissues[51].

2.4.3. *Candida albicans*

The dimorphic fungus *Candida albicans* is both a commensal and opportunistic pathogen of human. The predisposing factors for candidiasis include immunosuppressive, antibiotic cytotoxic therapies, the presence of intravenous catheters and indwelling devices, very low birth weight, AIDS, diabetes and drug abuse[52]. In addition *C. albicans* has been examined as a possible allergen in allergic diseases such as bronchial asthma[53]and atopic dermatitis[54].

2.4.3.1. Cell wall mannoproteins

The cell wall of *C.albicans* may be envisaged as a dynamic and plastic, multilayered structure located external to the plasma membrane. The presence of the wall is essential aspect of the biology and pathogenicity of *C. albicans*[55].

The major components (80 to 90%) of the cell wall of *C.albicans* are carbohydrates. Mannan-a major component of carbohydrates in the cell wall of *C.albicons* – or polymers of mannose covalently associated with proteins to form glycoproteins, also referred to as Mannoproteins[56].

Savolainen *et al,* 1996 examined the reactivity of immunoglobulin E (IgE), IgG, and IgM antibodies to mannan by a radioallergosorbent test (RAST) and ELISA and to proteins (some of which are in the cell wall) by immunoblotting and concluded that the IgE titre rose early and was more useful for detection than were IgG and IgM titres. Also, RAST and immunoblotting analyses were both required for maximum sensitivity and specificity[57].

Several reports have indicated that IgE antibodies against yeast mannan, a major component of the carbohydrate in the cell wall of *C.albicns*, are seen in patients with atopic dermatitis and bronchial asthma[54,58].

2.4.3.2. Glycolytic enzymes

Glycolytic enzymes are abundant immunodominant antigens during *C.albicans* infections. *C.albicans* enolase (2-phospho-D-glycerate hydrolyse), phosphoglycerat kinas (PGK), alcohol dehydrogenase (ADH), pyruvatekinase (PYK), aldolase and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) have been described as a major allergens or immunogens during candidiasis [59].

Ishiguro *et al*, 1992 investigated the antigenic components of *C.albicans* to IgE antibodies specific to enolase, phosphoglyceratkinase and aldolase in the sera of patients who were positive for IgE antibodies against *C.albicans* in RAST[60].

2.5. Diagnosis of allergy

2.5.1. Skin test

Skin testing is most commonly used in the diagnosis of allergy. It is usually performed by the skin-prick method using concentrated allergen extracts or intradermal skin test (injection of allergen within the dermal layer). With skin-prick tests, a wheal diameter of 3mm is the most reliable cut-off point for differentiation between sensitized and non-sensitized individuals[61].

Antihistamines interfere with the development of wheal and flare reaction and should be stopped before immediate type skin testing. First generation antihistamines may be stopped two to three

days before testing, but the newer, second generation antihistamines can affect skin testing result for 3 to 10 days or longer[62]. Medication with antihistamine properties, such as anticholinergic agent, phenothiazine and tricyclic antidepressants also should be discontinued before skin testing[63].

2.5.2 Serodiagnosis of allergy

Rdioallergosorbent test (RAST) was the first widely employed method of detecting IgE antibodies in blood that are specific for a given allergen[64].

Western blotting or immunoblotting was another method to detect IgE in blood. In this method the binding sites of human IgE can be detected with a labelled anti-human IgE[65].

Enzyme-linked immunosorbent assay (ELISA) and ELISA inhibition is non-radioactive method based on the same principal to detect IgE depending on the assay design of inhibition method. It is possible to measure all allergen in crude extract or single allergen[66].

Several methods are available to detect antibodies (Abs) against antigens of *C.albicans* in serum. These methods rely on agglutination of yeast-phase Candida or reactions with extracts of Candida, namely, precipitin testing, counter immunoelectrophoresis and passive hemagglutination (PHA). Reaction of ^{125}I-labelled antiglobulin with anti-Candida Ab. Interpretation of data obtained

with these method is difficult because the procedure do not allow precise quantitation of the level of Ab reacting with a given antigen preparation. Moreover, the antigens recognized by Ab have not been identified because of poor characterization of the Candida extract used in these tests[67].

IgE anti-lipopolysaccharide was assayed by enzyme linked immunosorbent assay with monoclonal anti-human IgE[68].

2.6. Treatment of allergy

The fundamental principles for the treatment of allergic diseases are allergen avoidance, pharmacological treatment and immunotherapy (specific desensitisation).

Allergen avoidance required avoidance of each irritant such as smoke, dust, aerosols as well as animal dander, feathers, mold and house dust, these should be eliminated from the house. Masks and air filters also have a useful role to play, but, usually avoidance is difficult for the general atopic population[10].

Modern pharmaceutical chemistry has provided a lot of drugs that are more or less effective at various stages in the evolution of the anaphylactic reaction. This drug includes cromolyn sodium, which stabilizes membranes, prevents Ca^{2+} influx, and decreases or prevents mast cell degranulation. Corticosteroids, which block the

metabolic pathways involving arachidonic acid and have general anti-inflammatory effects, thus preventing late phase reaction.

Antihistamines, which compete with histamine for receptor sites, therapy decreasing or preventing immediate symptoms such s sneezing, itching and runny nose[30].

The allergen extracts was used as immunotherapy treatment for selected patients with certain IgE-mediated hypersensitivity[69]. The immunotherapy was introduced for first time to establish immunity against pollen as oral allergens[70]. This treatment provides some protection against the effects of natural allergenic exposure by providing regular administration usually by subcutaneous injection of a soluble extract of the relevant allergen administered in increasing amount until a maximum tolerated dose is reached[71], such repeated introduction by subcutaneous injections appears to cause a shift towards IgG production or to induce T-cell mediated suppression (possibly by a shift to the T_{h1} subset and IFN-γ production) that turn off the IgE production[72].

This process is often called desensitisation because it is possible to demonstrate reaction in sensitivity of target organ (eye, nose or bronchi) after challenge with the relevant allergen [73].

Chapter Three

Materials & Methods

Chapter Three
Materials and Methods

3.1. Study population

The investigated population comprised one hundred fifty patients attending the Centre of Asthma and Allergic Diseases in Basrah, during the period from July 2004 to November 2004. The patients were of both sexes (ninety eight females and fifty two males). Patient's age ranged between ten and sixty years. Patients were complaining of symptoms related to upper or lower respiratory tract disorders or conjunctivitis or pruritis.

In addition, a control group of one hundred individuals with no complaints of allergic diseases also included in the study.

3.2. Sampling procedure

Blood samples were collected from patients and control group and submitted for serodiagnosis.

3.3. Materials

3.3.1. Chemicals

I- Media

- Nutrient broth, OXOID, England.

II- Proteins

- Bovine serum albumine, Fluka, Germany.
- Lytacase, Sigma, U.S.A.
- Ovalbumine, Fluka, Germany.

- Papine, Fluka, Germany.
- Ribonuclease A, Fluka, Germany.

III- ELTSA Kits

- Total ELISA kit, Biomaghreb, Tunisia.
- Specific ELISA kit, Biomaghreb, Tunisia.
- Standard *Candida albicans* Disc (M5), CCM, Biomaghreb, Tunisia.

IV- Other Chemicals

- Phosphate buffer saline, BDH Chemicals, UK.
- G-75 sephadex, BDH chemicals, UK.
- Triss-buffer (hydroxyl methylaminomethan), BDH chemicals, UK.
- Phenol 90%, BDH chemicals, UK.
- Methanol, BDH chemicals, UK.
- Ethanol 96%, BDH chemicals, UK.
- Magnesium chloride, BDH chemicals, UK.
- 2-mercapatoethanol, BDH chemicals, UK.
- Sodium azide, Sigma, U.S.A.
- Glucose, OXOID, England.
- Yeast extract, OXOID, England.
- Peptone, OXOID, England.
- Sorbitol, Sigma, U.S.A.
- EDTA, OXOID, England.
- Triethanolamine, OXOID, England.

3.3.2. Instruments

- Refrigerated centrifuge, MSE, England.
- Incubator 37c°, Labsco, W.Germany.
- Shaker incubator, Bio-tek, U.S.A.
- Shaker water bath, Bio-tek, U.S.A.
- Universal Microplate Reader, ELX 800, Bio-tek, U.S.A.
- ELISA washer, ELX 50, Bio-tek, U.S.A.
- Freez dryer, Edward, Germany.
- PH meter, Fisher, 619A, U.S.A.
- Spectrophotometer, LKB, Sweden.

3.4. Methodology

3.4.1. Allergen preparation.

3.4.1.1. Bacterial Lipopolysaccharide (LPS)

I- Organism and culture conditions.

Escherichia Coli ATCC 25922 (obtained from central Health Laboratory) was cultivated in nutrient broth for 24 hours at 37c° with shaking[74].

II- Bacterial LPS extraction.

The bacterial LPS allergen was prepared according to method of Galanose *et al*, (1972)[75] as follow.

1- Bacterial cells were precipitated in cold centrifuge at 6000 rpm for 30 minutes.

2- The sediment was washed twice with normal saline, and then 300ml of distilled water was added after drying and incubated in shaker water bath at 70c° for 10 minutes.

3- 300ml of phenol 90% was added and shaken for 10 minutes at 70c°.

4- The mixture was left at room temperature and precipitated for half an hour at 6000 rpm in cold centrifuge.

5- The supernatant was dialysed against 1L D.W. for two days.

6- The extract was sterilized by using the millipore filter (0.45µm) and mixed with 300ml of cold methanol containing 3ml of (20% magnesium chloride in ethanol).

7- The solution was left for one hour and was precipitated by cold centrifuge at 7000 rpm for 30 minutes.

8- The sediment was soluted in 100ml of distilled water. 200ml of ethanol was then added.

9- Lyophilization to the extract was done by a lyophilizer.

3.4.1.2. *Candida albicans*

I- Organism and culture condition

C. albicans 3153 A (obtained from Central Health Laboratory) was used in these experiments. The strain was cultured onto GYEP (2% glucose, 0.3% yeast extract, and 1% peptone) agar plate for 48hr at 30c°, and then yeast cells from a single colony were inoculated into GYEP broth and were allowed to proliferate

for 24hr at 37c° with aeration on a reciprocal shaker at 100 strokes per minute[76].

II- Extraction of *Candida albicans*

The *Candida albicans* allergen was prepared according to the method of Ishiguro *et al* (1992)[60] as follows: -

1- Cells were grown at 37c° in GYEP broth, and then harvested by centrifugation at 4000 ×g for 5 minutes.
2- The sediment was washed twice in cold 10 mM sodium azide.
3- The cells were converted to spheroplast by suspension in 40ml of 50mm phosphate buffered saline (PBS) (pH 7.5) containing 1.4M sorbitol, 20mg of lytacase and 0.24 ml of 2-mercaptoethanol for 30 minutes at 37c°.
4- The mixture was centrifuged at 10000 ×g for 20 minutes.
5- The Pellet was homogenized in 10ml of 10mM triethanolamine (pH 7.2) containing 0.8 M sorbitol and 1 mM EDTA by 20 strokes with a shaker and centrifuged at 10000 ×g for 20 minutes to remove unbroken spheroplast and cells debris.
6- The supernatant was stored at 4c°.

III- Determination of protein content

The protein content of allergen extract was estimated according to Whitaker and Granum (1980) method[77]; Three millilitres of each extract were pipette in a quartz cuvattes. The absorbance value was measured spectrophotometrically at 235

and 280nm. The protein content in mg/ml was calculated by the following equation: -

$$\text{Protein content mg/ml} = \frac{A_{235} - A_{280}}{2.51}$$

IV- The purification and fractionation of protein extract on G-75 sephadex

Gel-filtration-liquid chromatography used to fractionate and purified the protein extract into molecules of different molecular weights by using G-75 sephadex according to the method of Leslie and Frank, 1976)[78], as follow:

1- Eight grams of dry sephadex (G-75) were swollen in about 120ml of 0.5M triss buffer pH 6.8, 1M HCL.

2- The gel poured into the column in vertical level using a glass rod to avoid any air bubbles and left to settle with opened outlet, the flow control value was opened and the water was allowed to enter the column.

3- Blue dextran stain at 1:100 w/v in water was added to the surface of the gel and effluent was collected into a graduate cylinder. The water was added to the surface of the gel and effluent was collected until the blue dye just appears, the collected liquid represents the void volume of the column.

4- The gel was equilibrated with 0.1 M triss buffer pH 6.8 equivalent to three times the void volume of the column.

5- The column was calibrated with standard molecular weight proteins; Ribonuclease A (13.7 KD), papine (23 KD), ovalbumine (45 KD), and Bovine serum albumin (67 KD), each one was applied individually, the volume of calibration solution should be 1-2% of total gel bed volume (VT); the elution volume (ve) for the calibration proteins was determined by spectrophotometer.

6- A calibration curve was fitted by measuring the elution volume (Ve) of several standard proteins, their corresponding distribution coefficient (kav) values, were calculated using the

following equation.

$$Kav = \frac{Ve - Vo}{Vt - Vo}$$

Where:

Kav = distribution coefficient

Ve = elution volume for the protein

Vo = column void volume = elution volume of blue dextran

Vt = total bed volume

The Kav values were plotted versus the logarithm of their molecular weight.

7- The extract was applied to the column at flow rate 21ml/hour. Fractions (3ml each) were collected in a sterile glass tubes and monitored for protein content spectrophotometrically at 280 nm.

8- Each major peak for extracts was determined by plotting the elution volume of protein extract versus the absorbance value at wave length 280nm. Each major peak was used as antigen in ELISA technique.

3.4.2. Enzyme Linked Immunosorbent Assay (ELISA)

3.4.2.1. Total IgE value estimation.

Total serum IgE was measured according to the instructions of Biomaghreb company.

1- Microtiter plate coated with mouse monoclonal IgE anti-human IgE was used to estimate the total IgE antibodies.

2- Assay buffer (100µl) was added to each well.

3- To the first and second wells of first vertical row, 20µl of control standard IgE (0µl/ml) was added.

4- To other six wells of first vertical and to the fourth wells of second vertical row, standard IgE at the concentration (2, 5, 20, 50, 200 and 500 IU/ml) were added.

5- To the last of the two wells of the second vertical row, 20µl of the control positive and negative serum were added.

6- Serum samples(20µl) were added to the rest of wells

7- The plate was covered with plastic film and homogenized by shaking at 300 rpm and incubated at 37c° for 90 minutes

8- Cover film was removed and the plate was washed with washing solution phosphate buffer saline (pH 7.4) containing 0.05% tween 20, this procedure was repeated three times.

9- Goat antihuman IgE alkaline phosphates conjugate (100µl) was added to each well. Then, the plate was covered with plastic film and incubated at 37c° for 90 minutes.

10- Cover film was removed and the plate was washed as in step 7.

11- Freshly prepared substrate solution, 100 µl was added to each well. The substrate solution consisted of para-nitro-phyenyl-phosphate (PNPP).

12- Cover film was applied and the plate was incubated at room temperature for 30 minutes in the dark.

13- The stopping solution (100µl) (2N NaOH) was added to each well.

14- The absorbance of each well was read at 405 nm using microplate reader.

3.4.2.2. Specific IgE value estimation using locally prepared and standard *Candida albicans* allergen

Specific IgE antibodies were measured according to the method of Biomaghreb company as follow:

1- The first and second wells of vertical row left empty for blanking.

2- Reference disc D_1 allergen (*Dermot pteron*) were added in the bottom of microtiter plate, started with third well of first vertical row to eight well of second vertical row.

3- A reference serum calibrator (A, B, C, D and H) in which IgE concentration (52.50, 17.50, 3.50, 0.70 and 0.35 Eu/ml) was added to the reference D_1 disc.

4- Filter paper disc were prepared, sterilized by autoclaving at 121 C° for 15 minutes and saturated with locally prepared *Candida albicans* allergen extracts. The protein content of these extracts were determined according to the protein content of the standard *C. albicans* allergen (Ag) which are produced by Biomaghred company.

5- The locally prepared antigen discs and standard discs of *C. albicans*_ allergen extracts were added to the bottom of the rest of wells.

6- Fifty µl of serum samples were added to all prepared and standard allergen discs. Then, the plate was covered with plastic film.

7- The microplate was gently shacked for 30 seconds (microplate shaker at 200 rpm) and incubated for 90 minutes at room temperature.

8- Cover film was removed and the plate was washed with washing solution. The procedure was repeated three times.

9- Fifty µl (50µl) of anti-IgE labelled with alkaline phosphate conjugate was added to each well except the blanking well. The plate was covered with plastic film and incubated at room temperature for overnight.

10- The plate was washed as in step 8.

11- Freshly prepared substrate solution (PNPP, 50µl) was added to all wells including the blank wells. Then, the plate was covered and incubated for 20 minutes in the dark at room temperature.

12- The stopping solution (200µl, 2N NaOH) was added to each well.

13- The absorbance of each well read spectrophotometrically with ELISA reader at 405 nm.

3.4.2.3. Chequer-board filtration ELISA using LPS allergens

1- The allergen extract was diluted in 0.15M PBS (pH 7.4) (washing buffer, 2.0g NaCl, 0.2g KCl, 1.15g $NaHP0_4$, 0.2g KH_2PO_4) containing 0.05% tween 20 in serial dilutions 1/10, 1/100, 1/1000, and 1/10000.

2- The first vertical row of the microtiter plate was left empty for blanking.

3- Across the plate (Horizontal row) 200µl per well of one LPS allergen dilution was added to the next row. The second antigen dilution was added and the same done for other two allergen dilutions. The plate was covered with cover film and incubated at 4c° for overnight.

4- Cover film was removed and the plate was washed and flooded with washing buffer. This procedure was repeated three times at 3-5 minutes intervals.

5- Three positive control sera were diluted to 2-flod dilution: 1/20, 1/40 and 1/80, 200µl from each dilution of each serum was added to the wells of each antigen dilution, starting from the second vertical row.

6- To the first well of the first vertical row. 200µl of sample diluents was added. This serving as the reagents blank, and was used for blanking the photometer.

7- To the other 3 wells of the first vertical row which were uncoated with antigen, 200µl of 1/20 diluted serum from the

above mentioned sera were loaded. These wells will give the values of the back ground if present. This step is applied in the subsequent tests to detect the values of the back ground in each run.

8- Cover film was applied and plate incubated at 37c° for 2 hours.

9- The plate was washed as in step 4.

10- The conjugate 100µl at dilutions of 1/100, 1/200, 1/400,1/600, 1/800and 1/3200 were added to the tested wells, the blanking well and to the three serums loaded wells.

11- Cover film was applied and the plate was incubated at 37c° for 30minutes.

12- Cover film removed and the plate was washed as in step 4.

13- Freshly prepared substrate solution of Para-nitro-phenyl-phosphate (PNPP), 100µl was added to each well mentioned in step 10.

14- Cover film was applied and the plate was incubated at 37c° for 30 minutes in the dark.

15- The stopping solution 100µl (2N-NaOH) was added to each well as mentioned in step 10.

16- The absorbance of each of these wells was read at 492 nm using microplate reader after blanking the photometer on the blanking well.

3.4.2.3.1. Test to confirm the utility of the applied ELISA

I- Pilot study

This test was performed to check if the antigen dilution selected from CB-ELISA is optimal dilution or not. The highest antigen dilution that give on optical density reading of (1.0) with the selected serum dilution of positive samples and a reading of $\leq$ 0.1 with the same dilution of the negative samples regarded as optimal antigen dilution[79]. This mean, any antigen dilution selected from CB-ELISA is able to achieve this target in the pilot studies, it will be the optimal antigen dilution.

This test was used on thirty-eight (38) randomly chosen samples from the samples included in this study.

II- Testing the reproducibility of the applied ELISA

Randomly chosen samples, which have been previously tested, were subjected to repeat test in the presence of the same environmental condition with application of the same antigen and antibody dilution of the first test.

III- Detection of the true negatives

Negative samples found in this study were tested again at serum dilution of 1/10, in order to find out if these samples contain low antibody level that can not be detected at higher serum.

IV- Estimation of the negativity cut off

The negativity cut off was determined according to the method of Difelice *et al*, 1994[80]. As follow: -

Ten-serum samples from normal subjects were tested as negative control. The mean OD 492 value plus 3 SDS of the mean, calculated for the normal groups regarded as negativity cut off.

3.4.3. Statistical methods

For the determination of statistical significance among the males and females in different age groups regarding the number of the patients who had positive ELISA result. The statistical analyses were made using the qi-sequre (X^2) test.

Chapter Four

RESULTS

Chapter Four
Results

4.1 Purification of Potential allergens

4.1.1 Bacterial LPS extraction

100 µg was extracted from *E. coli* (ATCC 25922) as previously mentioned in (subsection 3.4.1.1) after lyophilization.

4.1.2 Candida albicans extracts

The protein extracts from *C.albicans* was purified and fractionated by gel filtration using sephadex G-75. On fractionating *C. albicans* proteins, three major peaks were observed (fig.1). The protein molecular weight and concentration of each major peaks of *C.albicans* extract with final recovery of the purified protein in relation to the original protein content were reported on (Table 1).

4.1.2.1 Estimation of the molecular weight of *C.albicans* purified protein

The molecular weight of purified antigens was estimated by gel filtration, using the blue dextran (2000 KD) for the determination of the void volume (vo) of the column. The highest absorbance value of the standard protein was measured spectrophotometerically. Gel filtration calibration curve was fitted by measuring the elution volume of ribonulease A (13.7 KD), papine (23 KD), ovalbumine (45 KD) and bovine serum albumine (67 KD). The Kav value were calculated and plotted the logarithm of their molecular weight (Fig. 2).

Table 1: The protein content and the molecular weight of purified protein extracts of *C.albicans*

C.albicans allergens	Protein concentration %	Molecular weigh (KD)
Peak I	0.03	46
Peak II	0.18	43
Peak III	0.02	37

4.2 ELISA Results

4.2.1 Estimation of total IgE value

According to the IgE values, there were three types of allergy, allergy not probable (< 20 μg/ml), allergy questionable (20-100 μg/ml) and allergy very probable (> 100 μg/ml) (table 2). One hundred thirty nine patient had positive total IgE ELISA results. These patients then tested with locally prepared potential allergen and standard *C.albicans* allergens as well as locally prepared bacterial LPS potential allergen.

Table (2): Estimation of total IgE value

Total IgE values (μg/ml)	No. and percentage of patients examined	Type of allergy
< 20	11 (7.4)	Allergy not probable
20-100	55 (36.6)	Allergy questionable
> 100	84 (56.0)	Allergy very probable

4.2.2 Prevalence of *C.albicans* allergens

Table (3 and 4) show the rate of ELISA positive results of specific IgE in one hundred thirty nine patient who examined with locally prepared *C. albicans* potential allergen (Peak I, II and standard) and peak III *C.albicans* potential allergens. The highest rate of specific IgE ELISA

results were observed in males and females of first and second age groups as follow, first age group males (88.8% and 55.5%) and females (100% and 78.9%). Second age group males (75% and 37.5%) and females (89.4% and 73.6%) respectively. The rate of distribution was decreased gradually in the third, fourth and fifth age groups in both sexes.

Also, the highest rate of positive IgE ELISA results was observed in females in comparison with males.

On the other hand, table (3) shows that, there is no difference in prevalence rate between the results of positive specific IgE ELISA in patients examined with locally prepared *C.albicans* potential allergen (peak I and II) and standard *C.albicans* potential allergen (peak I and II) and standard *C.albicans* allergen. While there is a difference in prevalence rate of positive IgE ELISA results in patients examined with locally prepared *C.albicans* potential allergen (Peak III) and standard *C.albicans* allergen (table 4).

Data of table (5) show the mean ± SD of the OD values of positive serum samples were tested with locally prepared and standard *C.albicans* peak I, II and III allergens using IgE based ELISA. In this table, it is obviously shown that there is no difference between age groups or males and females concerning their OD values.

Table (7) shows that the most common atopic disorders of *C.albicans* peak I and II allergy is pruritis (47.6%) followed by asthma (28.0%).

4.2.3 Results of chequer – board ELISA

The final dilations of *E. coli* LPS potential allergen, sera and conjugate were 1/1000, 1/20 and 1/100 respectively, while the back ground and negative cut off optical density (OD) values of IgE based ELISA were < 0.070 and 0.039 respectively.

4.2.3.1 Prevalence of lipopolysaccharide allergy

Table (6) shows the rate of positive ELISA results in 139 patients examined with LPS potential allergen. The highest rates were observed in males and females of the first and second age groups as follow, first age group (10-20 years) in both males (44.4%) and females (93.8%) followed by the second age group (21-30 years) males (37.5%) and females (78.9%), the rate of distribution was decreased gradually in the third, fourth and fifth age groups in both sexes. Also, the highest rate of positive IgE ELISA results was observed in females in comparison with males.

2.2.4 Specific IgE results of control

The specific IgE ELISA result of 139 patients and 100 control with negative responses to locally prepared *C.albicans* and LPS potential allergen and standard *C.albicans* allergen were shown in figures (3,4,5).

Table (7): The most common atopic disorders of *C.albicans* (Peak I and II) and LPS allergy in allergic patients

	Atopic disorders				Total
	Rhinitis no. (%)	Conjunctivitis no. (%)	Asthma no. (%)	Pruritis no (%)	
C.albicans allergy	19 (17.4)	7 (6.5)	30(28.0)	51 (47.6)	107
LPS allergy	11(15.1)	6 (8.2)	36 (49.3)	20 (27.4)	73

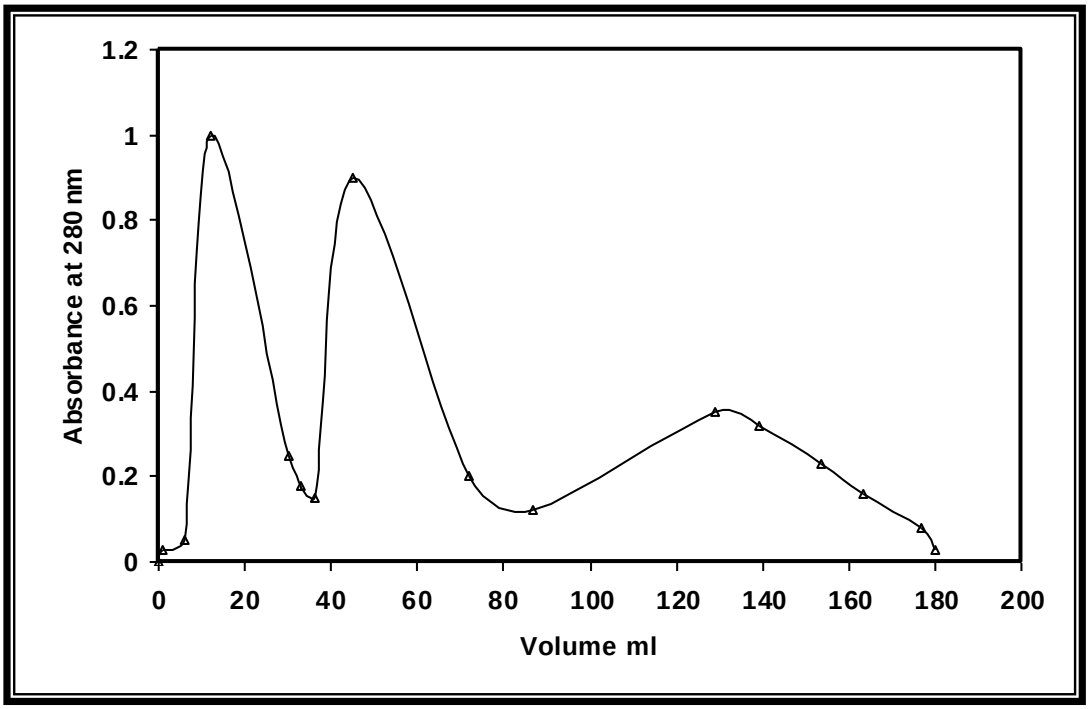

Fig. (1): Elution profile of *C. albicans* antigens

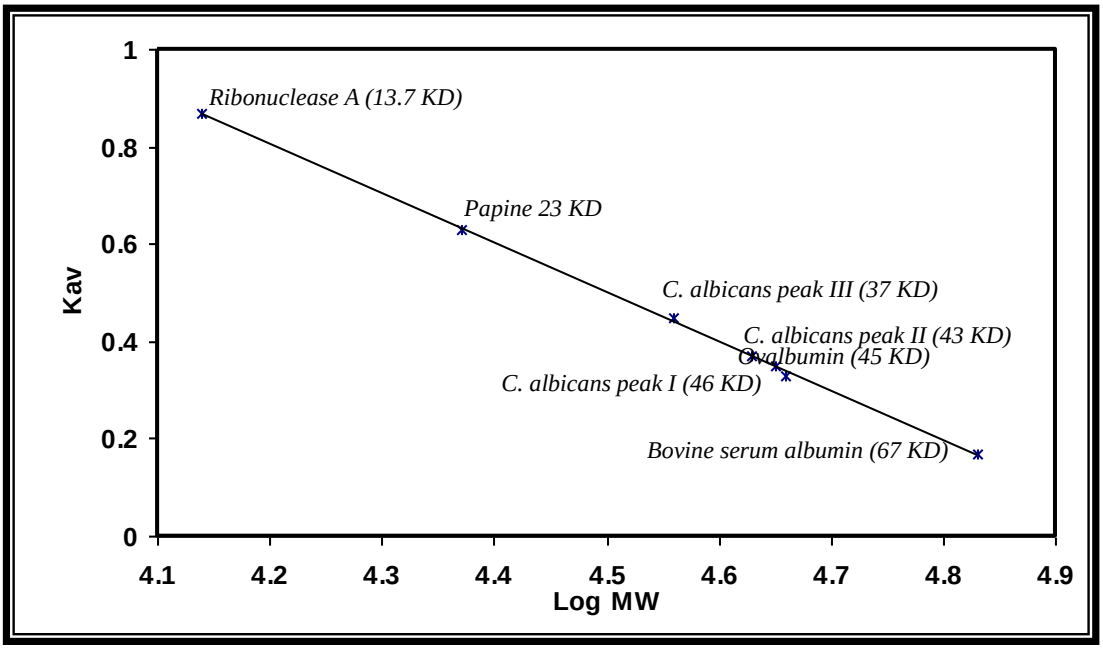

Fig. (2): The calibration curve of protein extract using some standard protein for estimation of protein molecular weight.

Table (3): Comparison of Specific IgE ELISA results in patients examined with locally prepared *C.albicans* potential allergen (Peak I and II) and standard *C.albicans* allergen

Age group (years)	Male				Female				Total
	Examined no.	Specific IgE Peak I no. (%)	Specific IgE Peak II no. (%)	Specific IgE S.A. no. (%)	Examined no.	Specific IgE Peak I no. (%)	Specific IgE Peak II no. (%)	Specific IgE S.A. no. (%)	
10-20	9	8(88.8)	8(88.8)	8(88.8)	16	16(100)	16(100)	16(100)	25
21-30	8	6(75.0)	6(75.0)	6(75.0)	19	17(89.4)	17(89.4)	17(89.4)	27
31-40	15	9(60.0)	9(60.0)	9(60.0)	29	25(86.2)	25(86.2)	25(86.2)	44
41-50	11	4(36.3)	4(36.3)	4(36.3)	27	20(74.1)	20(74.1)	20(74.1)	38
51-60	1	0	0	0	4	2(50.0)	2(50.0)	2(50.0)	5
Total	44	27	27	27	95	80	80	80	139

S.A. Standard allergen

Table (4): Comparison of Specific IgE results in patients examined with locally prepared *C.albicans* potential allergen (Peak III) and standard *C.albicans* allergen

Age group (years)	Male			Female			Total
	Examined. no.	Specific IgE Peak III no. (%)	Specific IgE S.A no. (%)	Examined no.	Specific IgE Peak III no. (%)	Specific IgE S.A no. (%)	
10-20	9	5 (55.5)	8 (88.8)	16	15(78.9)	16(100)	25
21-30	8	3 (37.5)	6 (75.0)	19	14(73.6)	17(89.4)	27
31-40	15	4 (26.6)	9 (60.0)	29	20(68.9)	25(86.2)	44
41-50	11	1 (9.1)	4 (36.3)	27	8(29.6)	20(74.1)	38
51-60	1	0	0	4	1(25.0)	2(50.0)	5
Total	44	13	27	95	58	80	139

S.A. Standard allergen

Table (5): ELISA results in patients with positive specific IgE responses to *C.albicans* potential allergens (Peaks I, II and III) and standard *C.albicans* allergen

Age group (years)	Male				Female			
	Peak I Mean ± S.D	Peak II mean ± S.D	Peak III mean ± S.D	Standard mean ± S.D	Peak I Mean ± S.D	Peak II mean ± S.D	Peak III mean ± S.D	Standard mean ± S.D
10-20	0.562 ± 0.386	0.988 ± 0.877	0.878 ± 0.728	0.850 ± 0.734	1.903 ± 1.448	2.211 ± 1.421	1.326 ± 0.888	2.213 ± 1.670
21-30	0.355 ± 0.056	0.891 ± 1.047	0.380 ± 0.077	0.548 ± 0.348	12.196 ± 6.844	11.248 ± 6.477	9.866 ± 5.042	13.057 ± 7.311
31-40	0.639 ± 0.464	0.985 ± 0.788	0.828 ± 0.730	0.800 ± 0.625	5.869 ± 4.578	5.273 ± 4.703	4.932 ± 3.595	6.466± 4.919
41-50	0.663 ± 0.316	1.630 ± 1.105	Nill	1.018 ± 0.536	1.839 ± 1.435	2.181 ± 1.478	2.195 ± 1.784	2.746 ± 2.188
51-60	Nill	Nill	Nill	Nill	5.082 ± 0.453	6.839 ± 2.188	Nill	6.266 ± 0.349

S.D. Standard deviation

Table (7): Rate of ELISA results in 137 Patients tested with LPS potential allergen

Age group (years)	Male		Female		Total
	Examined no.	Specific IgE LPS no. (%)	Examined no.	Specific IgE LPS no. (%)	
10-20	9	4 (44.4)	16	15 (93.8)	25
21-30	8	3 (37.5)	19	15 (78.9)	27
31-40	15	5 (33.3)	29	18 (62.1)	44
41-50	11	3 (27.3)	27	10 (37.0)	38
51-60	1	0	4	0 (0.0)	5
Total	44	15	95	58	139

LPS Lipopolysaccharide

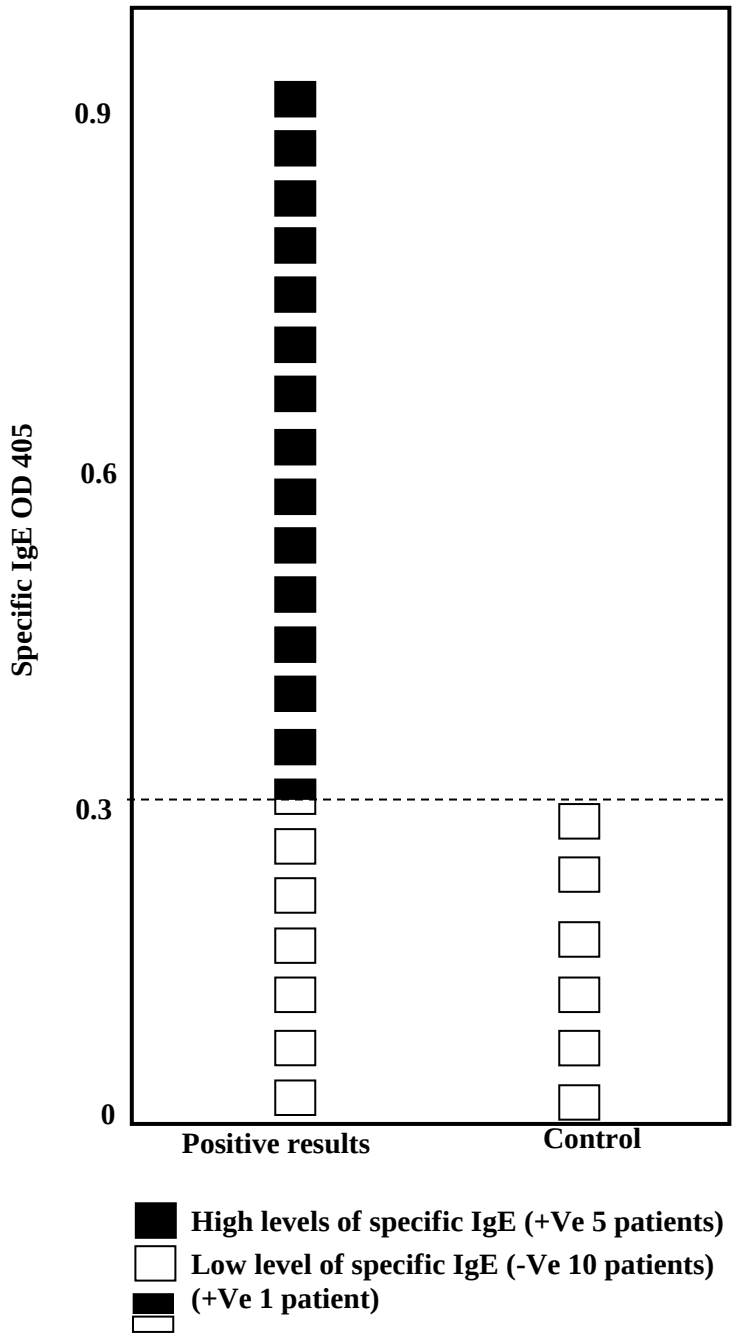

Fig.3 Positive, negative and control results of 239 individuals tested with locally prepared *C. albicans* allergens (peak I and Π) and standard *C. albicans* allergen.

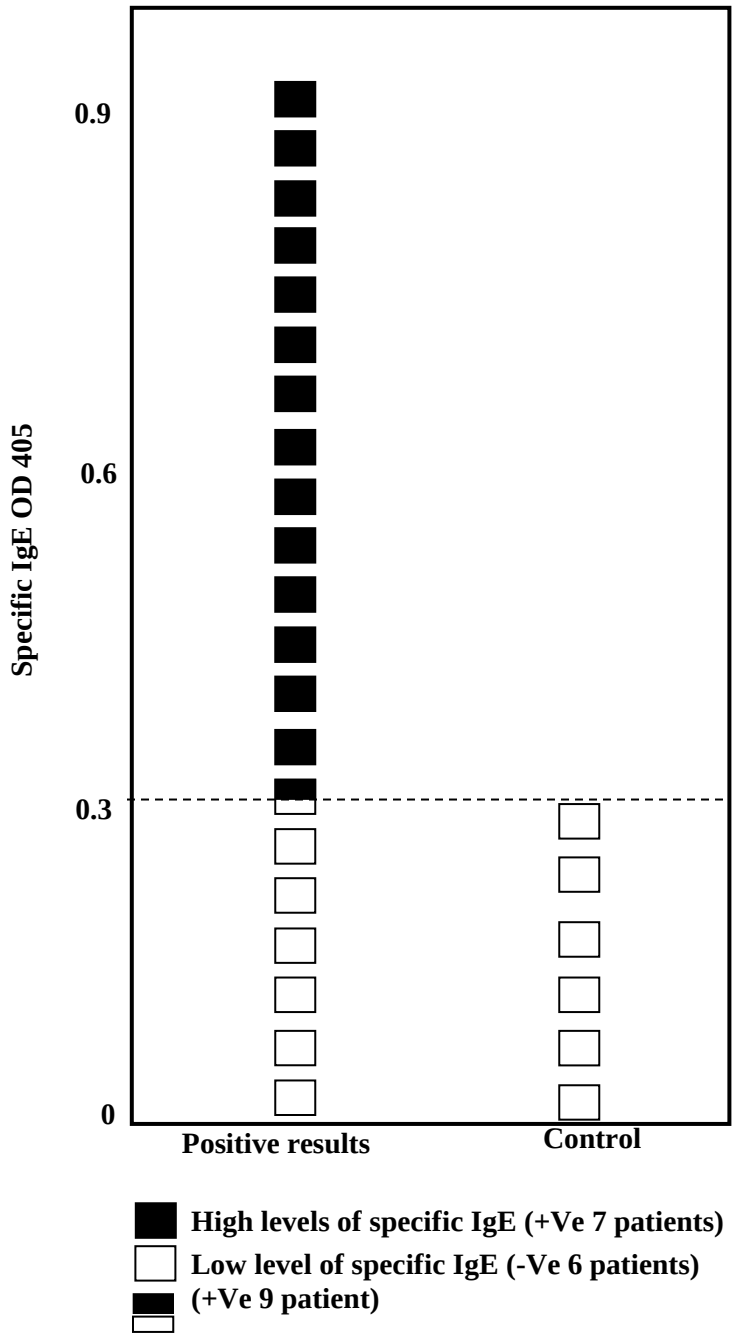

Fig.4 Positive, negative and control results of 239 individuals tested with locally prepared *C. albicans* allergen peak III.

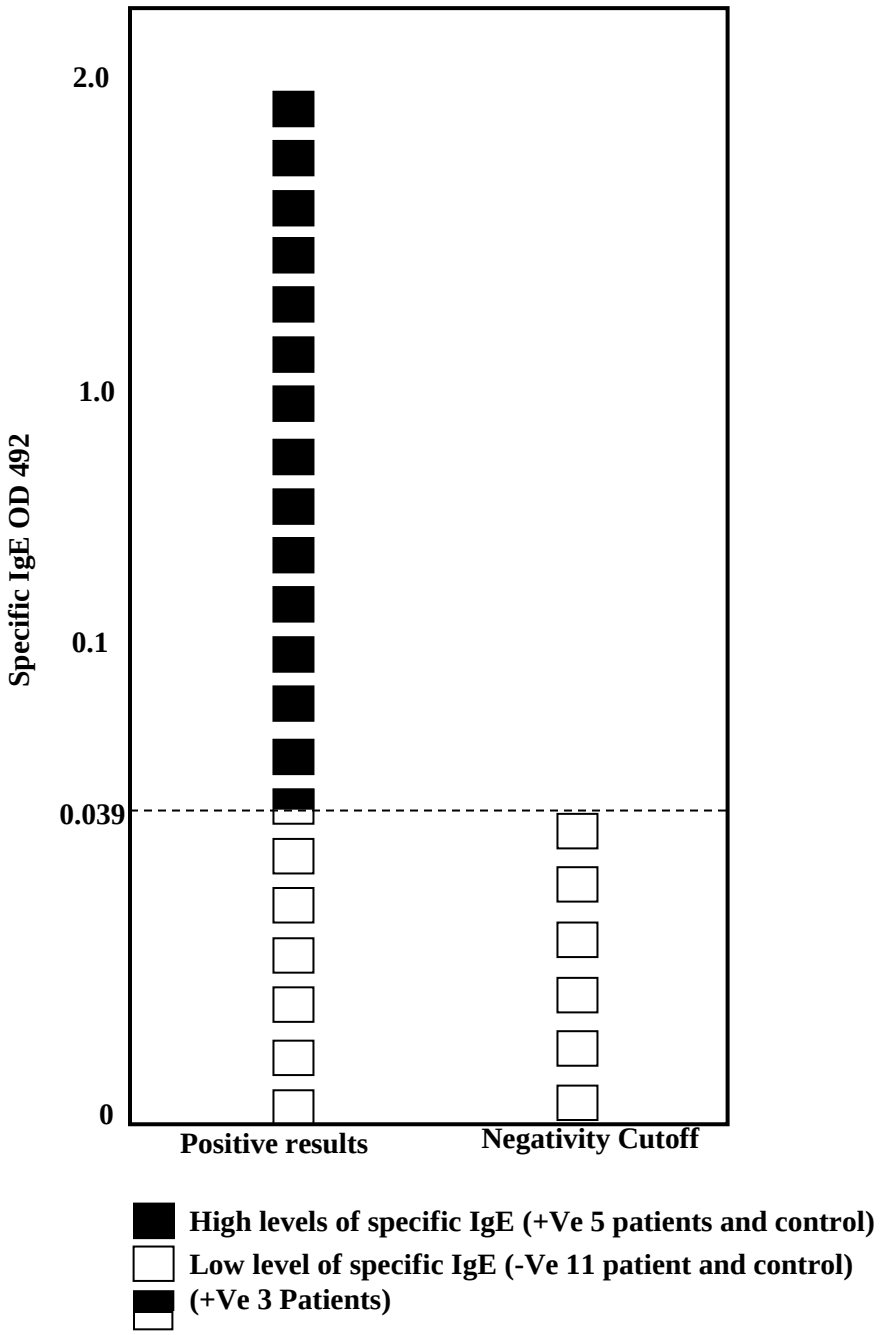

Fig.5 The negativity cutoff of IgE in 239 patients and control group who were tested with LPS potential allergen.

Chapter Five

Discussion

Chapter Five
Discussion

5.1 Purification of potential allergens

In recent years, many allergens of different sources have been investigated, purified and characterized immunochemically and biochemically. Gel filtration analysis of protein extract of *C.albicans* demonstrated three peaks, two of them, which represent the major potential allergens, with molecular weight of 46 KD and 43 KD. While the third peak with molecular weight of 37 KD represents a minor potential allergen. This result was in line with the results of Groot *et al* (1988)[81] and Shen *et al* (1989)[82], who reported that the major allergen is an antigen to which more than 50% of allergic patients have IgE antibodies in their sera. In addition, the major reported antigens were the 43 KD protein which reacted with 77% of the serum samples and the 46 KD protein which reacted with 77% of the serum samples were located in the cytoplamic fractions of *C. albicans*[60].On other hand, the molecular weight of the eluted protein was in line with the finding of Dreborg *et al*, 1986[83]; Marsh and Norman, 1988[39], who reported that the allergen molecules usually has a molecular weight of 5000-70000 daltons.

Enterobacteriaceae have a complex antigenic structure. The heat stable somatic O (Lipopolysaccharide) antigen is the most

external part of the cell wall. It consists of repeating units of polysaccharide [84]. In this study four serial dilutions were used. The optimum dilution was 1/1000 g/ml (1µg/ml) which was used to detect IgE antibodies specific to LPS allergen in allergic patient. This result was in agreement with Dipiro *et al*, 1994[68] who reported that 1µg/ml of LPS allergen was added to wells of microtiter plate in ELISA test.

5.2 Prevalence of *Candida albicans* allergy

The yeast *C. albicans* is a common commensal of the human oral, vaginal mucosae and gastrointestinal tract. It causes not only opportunistic infection in immunocompromised patients but also allergic reactions in people sensitized to *C. albicans* [8].

The rate of patients with positive specific IgE ELISA test for *C. albicans* potential (peak I and II) was 76.98%. This result was in line with Ishiguor *et al* study[60], who found that the rate of patients with positive responses to *C. albicans* allergen was 77%. While this result was lower than the rate of Kanbe *et al*,1996[76], who found that the 2-meracaptoethanol (2-ME) extract obtained from *C. albicans* cells by extraction with 2-ME, react with almost all sera (98%) from patients, who were positive for IgE antibodies to *C. albicans*.

There was a significant differences ($P < 0.05$) among age groups of patients tested with *C. albicans* (Peak I, II and III) and

standard *C. albicans* allergen, regarding the rate of patients who had a positive responses to these allergens, this result is in agreement with that reported by Hattevig *et al*, 1992[85], who found that the immune responses were acquired progressively during childhood, peaking between 15 and 25 years declining gradually. Also, there is a significant difference ($P<0.05$) regarding the rate of positive responses among males and females examined with *C. albicans* allergen and this finding is supported by Knauer and Adkinson, 1983, who found a significant association between the number of the yeast isolated and the symptoms of pruritis and signs of abnormal vaginal discharge. Those investigators had also shown that, the use of hormonal contraceptives and the stages of menstrual cycle, are predisposing factors to *Candida* overgrowth [8]. Vaginal carriage of yeasts is more prevalent in pregnant than in non pregnant women, which means that the physiological factors associated with pregnancy predispose the vaginal epithelia for colonization by Candida [86].

On other hand, there is no difference between the locally prepared *C. albicans* (Peak I and II) potential allergens and the standard *C. albicans* allergen regarding the OD value in ELISA testing. These results were in agreement with the results of Oscar *et al*, 1991[87] and Martin 1998 *et al* [88], who reported that the methods of antigen preparation did not effect allergenic activity.

5.3 Prevalence of lipopolysaccharide allergy

The allergenic activity of the lipopolysaccharide allergen was determined by ELISA testing. There is a clear evidence that this extract is the most clinically important allergen. This finding is in line with Rylander *et al*, 1985[89]. Who reported that endotoxine, a cell wall component, is often present in high concentration in organic dusts as well as in air pollution.

There is a significant difference ($P < 0.05$) among age groups of patients examined with LPS allergen concerning patients who had positive ELISA results, these results are in agreement with that reported by Hatteving *et al*, 1992[85], previously mentioned in subsection (5.2). In addition, asthma is an increasing cause of morbidity and mortality in the world particularly among children [90].

There is a significant difference ($P < 0.05$) regarding the rate of positive responses between males and females examined with LPS allergen, this finding is in line with Netea *et al*, 1998[91] and Kline *et al*, 1999[92], who reported that women were more likely to develop airflow obstruction after the inhalation of lower doses of LPS.

There are a number of acquired and genetically determined differences between the sexes. For example, women have a lowered cardiovascular mortality than men do, at least in part, because of differences in serum cholesterol levels, women have

higher levels of high-density lipoprotein (HDL) cholesterol and Lower levels of low density lipoprotein (LDL) cholesterol than do men, LDL can bind LPS and diminishes LPS-stimulated cellular inflammation.

When a sensitized person is exposed to allergic materials, this leads to release of inflammation mediators and the development of allergic symptoms. The most common atopic disorder to LPS allergen according to this study is asthma (49.3%) and this result was in line with the finding of Michel *et al*, 1989[(6)] who reported that inhalation of LPS can induce bronchoobstruction in asthmatic patients and an increase of their histamine bronchial reactivity. On the other hand, the explanation of the LPS- induces bronchoobstructive reactions include an action on bronchial cells, such as mastocytes and macrophages. Indeed, inhalation of endotoxines of *E. coli* in guinea pigs induced the production of a platelet–activating factor from alveolar macrophages, which is a very potent bronchoconstrictor in human[(93)].

The most common atopic disorder to *C. albicans* allergens is pruritis (47.6%) and this finding is in line with Nermes *et al*, 1994[(58)] who reported that the IgE antibodies specific to *C. albicans* allergens in patients with atopic dermatitis, asthma, and allergic rhinitis, they showed that the IgE antibodies to such allergens were mainly found in patients with atopic dermatitis rather than patients with respiratory allergy.

Conclusions

1. The locally prepared *Candida albicans* allergens and *Escherichia coli* lipopolysaccharide show allergenic reactivity and can be used in serodiagnosis of allergy.
2. There is no difference between locally prepared *C. albicans* Peak I and II, so we can use any one of them in the serodiagnosis of *C. albicans* allergy, and there is no difference between these two peaks and standard *C. albicans* allergen.
3. The locally easily prepared *C. albicans* and *E coli* lipopolysaccharide are less costly than commercial prepared allergen.

Recommendations

1. Exposure to *C. albicans* and LPS allergens must be avoided because they are important causes of allergic diseases, especially asthma.
2. Recombinant allergens could reveal cross reaction structures in various allergens sources.
3. *Candida albicans* cell wall mannan should be isolated and studied.
4. Further studies concerning the relationship between the OD value and different clinical manifestation of allergy.

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Appendixes

Appendix (1):Optical density (OD) values of patients examined with *Candida albicans* allergen (peak I) using IgE based ELISA.

Patient No.	OD value IgE	Patient No.	OD Value IgE	Patient No.	OD value IgE	Patient No.	OD Value IgE	Patient No.	OD value IgE	Patient No.	OD Value IgE
1	0.415	25	14.004	49	0.344	73	1.566	97	0.314	121	0.893
2	0.425	26	0.437	50	20.077	74	0.574	98	2.371	122	0.921
3	0.313	27	3.639	51	0.531	75	21.319	99	0.388	123	2.999
4	0.310	28	0.263	52	10.554	76	9.344	100	22.532	124	1.588
5	0.355	29	1.435	53	0.350	77	0.050	101	0.568	125	1.424
6	0.033	30	0.188	54	8.327	78	16.997	102	4.629	126	0.311
7	2.856	31	0.196	55	0.922	79	2.102	103	3.935	127	0.633
8	0.368	32	0.243	56	1.032	80	0.141	104	0.288	128	0.523
9	0.631	33	0.160	57	0.963	81	0.107	105	0.680	129	0.359
10	0.110	34	3.535	58	0.347	82	0.248	106	12.536	130	6.817
11	0.298	35	11.622	59	0.385	83	1.522	107	4.557	131	3.262
12	0.441	36	1.424	60	4.761	84	0.011	108	17.992	132	4.683
13	0.245	37	0.225	61	0.188	85	0.315	109	5.212	133	2.639
14	0.277	38	0.597	62	0.200	86	0.123	110	0.788	134	2.856
15	0.343	39	0.342	63	0.984	87	2.349	111	0.920	135	0.444
16	0.252	40	0.136	64	0.206	88	18.392	112	0.709	136	3.975
17	0.347	41	0.143	65	3.902	89	0.410	113	0.818	137	8.740
18	0.297	42	0.202	66	10.656	90	6.816	114	1.777	138	12..344
19	0.214	43	0.122	67	0.494	91	6.084	115	9.233	139	1.585
20	0.267	44	0.187	68	4.673	92	3.622	116	11.001		
21	0.312	45	0.948	69	18.382	93	0.639	117	6.535		
22	0.375	46	5.403	70	3.129	94	0.162	118	2.102		
23	3.656	47	16.741	71	1.289	95	4.386	119	7.554		
24	8.974	48	0.272	72	0.799	96	0.276	120	3.667		

Appendix(2):Optical density (OD) values of patients examined with *Candida albicans* allergen (peak II) using IgE based ELISA.

Patient No.	OD value IgE	Patient No.	OD value IgE	Patient No.	OD value IgE	Patient No.	OD value IgE	Patient No.	OD value IgE	Patient No.	OD value IgE
1	0.387	25	10.210	49	3.000	73	1.180	97	0.344	121	0.324
2	0.311	26	0.732	50	20.252	74	0.853	98	1.653	122	0.465
3	0.306	27	0.481	51	0.761	75	20.482	99	0.420	123	4.246
4	0.326	28	0.098	52	7.392	76	10.308	100	14.369	124	0.920
5	0.462	29	2.731	53	1.922	77	0.059	101	0.681	125	1.467
6	0.207	30	0.108	54	8.775	78	15.642	102	3.763	126	0.321
7	3.811	31	0.029	55	2.132	79	3.119	103	4.396	127	0.450
8	0.395	32	0.201	56	3.176	80	0.348	104	0.105	128	0.844
9	0.421	33	0.198	57	1.894	81	0.153	105	0.435	129	0.329
10	0.195	34	5.251	58	0.973	82	0.241	106	10.844	130	6.378
11	0.182	35	14.722	59	0.961	83	4.760	107	4.066	131	2.41
12	0.322	36	3.001	60	5.292	84	0.241	108	15.000	132	3.955
13	0.211	37	0.222	61	0.218	85	0.592	109	4.027	133	2.777
14	0.189	38	0.959	62	0.239	86	0.199	110	1.239	134	4.000
15	0.685	39	1.203	63	2.102	87	3.488	111	1.001	135	0.525
16	0.242	40	0.151	64	0.183	88	20.806	112	2.316	136	3.209
17	0.351	41	0.051	65	3.909	89	0.432	113	0.675	137	6.752
18	0.271	42	0.292	66	7.325	90	4.819	114	1.543	138	10.860
19	0.287	43	0.001	67	0.631	91	6.912	115	6.430	139	2.416
20	0.255	44	0.204	68	4.321	92	1.459	116	10.811		
21	0371	45	2.862	69	16.555	93	0.402	117	6.764		
22	0.872	46	8.387	70	3.210	94	0.173	118	1.999		
23	1.762	47	19.195	71	2.931	95	3.762	119	9.841		
24	6.684	48	0.194	72	0.958	96	0.088	120	3.685		

Appendix(3):Optical density (OD)values of patients examined with *Candida albicans* allergen (peak III) using IgE based ELISA.

Patient No.	OD value IgE	Patient No.	OD value IgE	Patient No.	OD value IgE	Patient No.	OD Value IgE	Patient No.	OD value IgE	Patient No.	OD Value IgE
1	0.198	25	9.236	49	0.203	73	1.901	97	0.199	121	0.273
2	0.205	26	0.456	50	14.189	74	0.293	98	0.936	122	0.558
3	0.302	27	0.297	51	0.259	75	17.777	99	0.200	123	3.959
4	0.266	28	0.259	52	3.123	76	11.312	100	10.312	124	1.157
5	0.312	29	1.983	53	0.398	77	0.009	101	0.498	125	0.255
6	0.201	30	0.207	54	4.812	78	13.974	102	1.772	126	0.293
7	2.101	31	0.288	55	0.279	79	3.844	103	2.911	127	0.199
8	0.399	32	0.196	56	0.299	80	0.279	104	0.116	128	0.233
9	0.234	33	0.252	57	0.741	81	0.199	105	0.555	129	0.350
10	0.198	34	3.713	58	0.229	82	0.267	106	6.298	130	5.778
11	0.251	35	10.592	59	0.294	83	3.922	107	0.291	131	2.901
12	0.261	36	0.430	60	0.232	84	0.290	108	0.250	132	1.873
13	0.211	37	0.214	61	0.297	85	0.382	109	6.190	133	1.894
14	0.194	38	0.643	62	0.110	86	0.194	110	0.393	134	2.893
15	0.432	39	1.003	63	0.990	87	0.111	111	0.219	135	0.291
16	0.229	40	0.146	64	0.139	88	15.378	112	2.009	136	3.118
17	0.361	41	0.179	65	1.817	89	0.549	113	0.185	137	6.315
18	0.103	42	0.266	66	5.685	90	4.032	114	0.966	138	0.198
19	0.216	43	0.106	67	1.266	91	7.226	115	5.775	139	0.244
20	0.110	44	0.206	68	1.203	92	0.897	116	0.293		
21	0.343	45	0.289	69	13.108	93	0.285	117	7.109		
22	0.668	46	8.303	70	3.001	94	0.183	118	0.297		
23	0.959	47	13.275	71	0.050	95	2.201	119	5.894		
24	5.777	48	0.148	72	0.277	96	0.239	120	3.003		

Appendix(4):Optical density (OD)values of patients examined with standard *Candida albicans* allergen using IgE based ELISA.

Patient No.	OD value IgE	Patient No.	OD Value IgE	Patient No.	OD value IgE	Patient No.	OD Value IgE	Patient No.	OD value IgE	Patient No.	OD value IgE
1	0.420	25	14.152	49	1.232	73	1.668	97	0.481	121	1.891
2	0.445	26	0.587	50	23.001	74	0.895	98	2.401	122	0.922
3	0.357	27	4.279	51	0.981	75	23.005	99	0.311	123	3.426
4	0.315	28	0.103	52	0.544	76	10.105	100	20.892	124	1.588
5	0.355	29	2.551	53	0.885	77	0.181	101	0.817	125	1.783
6	0.105	30	0.070	54	10.351	78	15.677	102	4.962	126	0.926
7	3.112	31	0.110	55	0.921	79	3.215	103	6.482	127	0.633
8	0.387	32	0.103	56	2.003	80	0.213	104	0.101	128	0.801
9	0.502	33	0.098	57	1.636	81	0.176	105	0.712	129	0.411
10	0.211	34	4.661	58	0.465	82	0.283	106	13.786	130	7.835
11	0.276	35	13.516	59	1.000	83	2.481	107	4.557	131	3.420
12	0.483	36	2. 997	60	6.019	84	0.152	108	17.983	132	4.521
13	0.253	37	0.212	61	0.091	85	0.376	109	5.881	133	2.892
14	0.282	38	0.900	62	0.210	86	0.228	110	1.460	134	4.686
15	0.363	39	0.905	63	1.101	87	3.455	111	1.601	135	0.821
16	0.251	40	0.010	64	0.123	88	20.401	112	2.400	136	4.209
17	0.351	41	0.133	65	3.909	89	0.762	113	0.991	137	9.101
18	0.290	42	0.220	66	11.111	90	5.921	114	1.483	138	12.381
19	0.214	43	0.110	67	0.853	91	7.110	115	9.421	139	2.615
20	0.269	44	0.222	68	4.777	92	3.622	116	12.012		
21	0.343	45	1.763	69	21.321	93	0.342	117	5.899		
22	0.375	46	6.513	70	3.128	94	0.181	118	2.487		
23	3.654	47	20.237	71	2.010	95	5.822	119	10.546		
24	8.942	48	0.001	72	0.958	96	0.233	120	3.550		

Appendix(5): Optical density (OD)values of patients examined with LPS allergen using IgE based ELISA.

Patient No.	OD value IgE	Patient No.	OD Value IgE	Patient No.	OD value IgE	Patient No.	OD value IgE	Patient No.	OD value IgE	Patient No.	OD value IgE
1	1.020	25	1.113	49	0.442	73	0.334	97	0.542	121	0.653
2	0.934	26	1.221	50	0.015	74	0.946	98	0.018	122	0.422
3	0.030	27	0.036	51	1.261	75	1.931	99	0.432	123	0.038
4	0.561	28	0.025	52	0.432	76	0.013	100	0.037	124	0.031
5	0.031	29	0.034	53	0.012	77	0.652	101	0.033	125	0.011
6	1.157	30	1.131	54	0.037	78	0.019	102	0.363	126	0.027
7	0.813	31	0.652	55	1.611	79	0.934	103	0.037	127	0.429
8	0.609	32	0.032	56	0.481	80	0.445	104	0.023	128	0.015
9	0.423	33	0.422	57	1.821	81	0.032	105	0.777	129	1.710
10	0.193	34	1.262	58	0.531	82	0.027	106	0.481	130	0.038
11	0.735	35	0.034	59	0.843	83	0.035	107	0.037	131	0.032
12	0.021	36	0.034	60	0.024	84	1.235	108	0.241	132	1.921
13	0.035	37	0.023	61	0.315	85	0.016	109	0.021	133	0.825
14	0.015	38	0.019	62	1.623	86	1.020	110	0.208	134	0.036
15	1.162	39	0.018	63	1.141	87	0.931	111	0.030	135	0.855
16	0.222	40	0.855	64	0.021	88	0.027	112	0.319	136	0.035
17	0.491	41	0.523	65	0.021	89	0.020	113	0.015	137	0.029
18	0.115	42	0.023	66	0.300	90	0.032	114	0.026	138	0.497
19	0.866	43	0.031	67	1.235	91	1.333	115	0.036	139	0.023
20	0.035	44	0.027	68	0.014	92	0.017	116	1.089		
21	0.863	45	0.012	69	1.015	93	0.084	117	0.026		
22	0.028	46	0.020	70	0.761	94	0.022	118	0.034		
23	0.417	47	0.032	71	1.765	95	0.037	119	0.022		
24	0.089	48	0.011	72	0.013	96	0.211	120	1.220		

الخلاصة

حضرت المستخلصات المستأرجة من خميرة المبيضة البيضاء بواسطة الاستخلاص ثم التنقية والتجزئة بواسطة الترشيح الهلامي، تم الحصول على ثلاثة قمم بأوزان جزيئية 37 و 43 و 46 كيلو دالتون. كذلك تم استخلاص وتنقية متعدد السكريد الشحمي من الجدار الخلوي لبكتريا الاشريكية القولونية واستخدم كمستأرج.

أجري فحص IgE ELISA العام على مائة وخمسين عينة من مصول المرضى المراجعين لمركز أمراض الحساسية والربو في البصرة و مئة من الأشخاص الأصحاء كمجموعة سيطرة تراوحت أعمارهم بين (10–60) سنة. أعطى 139 مريضا نتائج موجبة لفحص IgE ELISA العام. ثم أجري فحص IgE ELISA المتخصص لمستأرجات المبيضة البيضاء المحضرة محلياً (قمم 1، 2، 3) ومستأرج المبيضة البيضاء القياسي ومستأرج السكريد الشحمي لبكتريا الاشريكية القولونية المحضر محلياً على عينة مصول 139 مريضا.

لقد وجد فرق إحصائي معنوي بين الفئات العمرية للمرضى الذين أظهروا نتائج موجبة لفحص IgE ELISA المتخصص لكلا النوعين من المستخلصات، ولوحظت أعلى النسب في الذكور والإناث بعمر 10–30 سنة. وكذلك ظهر فرق إحصائي معنوي بين الإناث والذكور اعتماداً على نسبة النتائج الموجبة لفحص IgE ELISA المتخصص لهذه المستأرجات حيث ظهر أن نسبة النتائج الموجبة للإناث أعلى منها للذكور.

في هذه الدراسة كانت نسبة المصابين بالربو نتيجة تعرضهم لمستأرج متعدد السكريد ألشحمي أكثر شيوعا من أمراض فرط الحساسية الأخرى , بينما كانت الحكة الجلدية أكثر انتشارا من بقية أمراض الحساسية في المرضى الذين اظهروا نتائج موجبة لفحص ELISA IgE المتخصص لمستأرجات المبيضة البيضاء.

دور مستأرجات المبيضة البيضاء ومتعدد السكريد الشحمي للاشريكية القولونية كعامل مسبب للنوع الأول من فرط الحساسية

رسالة مقدمة إلى

مجلس كلية الطب – جامعة البصرة

وهي جزء من متطلبات نيل درجة الماجستير في

علوم الإحياء المجهرية الطبية

من قبل الطالب

وميض هاشم عباس

بكالوريوس طب وجراحة بيطرية

رجب 1426 هـ آب 2005