Immunological & Bacteriological aspects about Atopic Eczema Syndrome

1- Infantile and child hood groups were recorded a high prevalence 34.7% and 27.5% respectively than adult age group (37.9%). Males were highly affected with AD in age groups (1,4,5) , while females predominated male in groups (2,3). In general, the male: female ratio of infection with AD was (1:1.29) (P< 0.001). 2- Face and face & neck were the commonest sites of predilection of AD lesions (33.1 % and 20%) respectively . 3- A high correlations between family and personal histories were shown with AD. 4- Dry type of skin , sub acute pattern of disease course, and mild to severe degree of severity were predominant forms in AD patients. 5- The values of Hb, RBCs count and basophiles of AD patients were under the normal or control means , while values of ESR, WBCs count , lymphocytes count , eosinophiles and platelets count were higher than normal means . On other hands , values of differential neutrophils , lymphocytes and monocytes were within normal means (P< 0.001) . 6- The study showed a high significant elevation of various types of cluster of differentiation (CD): (CD3 ,CD4 , CD8 , CD19) .Also , all means of concentration of immunoglobulin (Ig) (IgA, IgG , IgM

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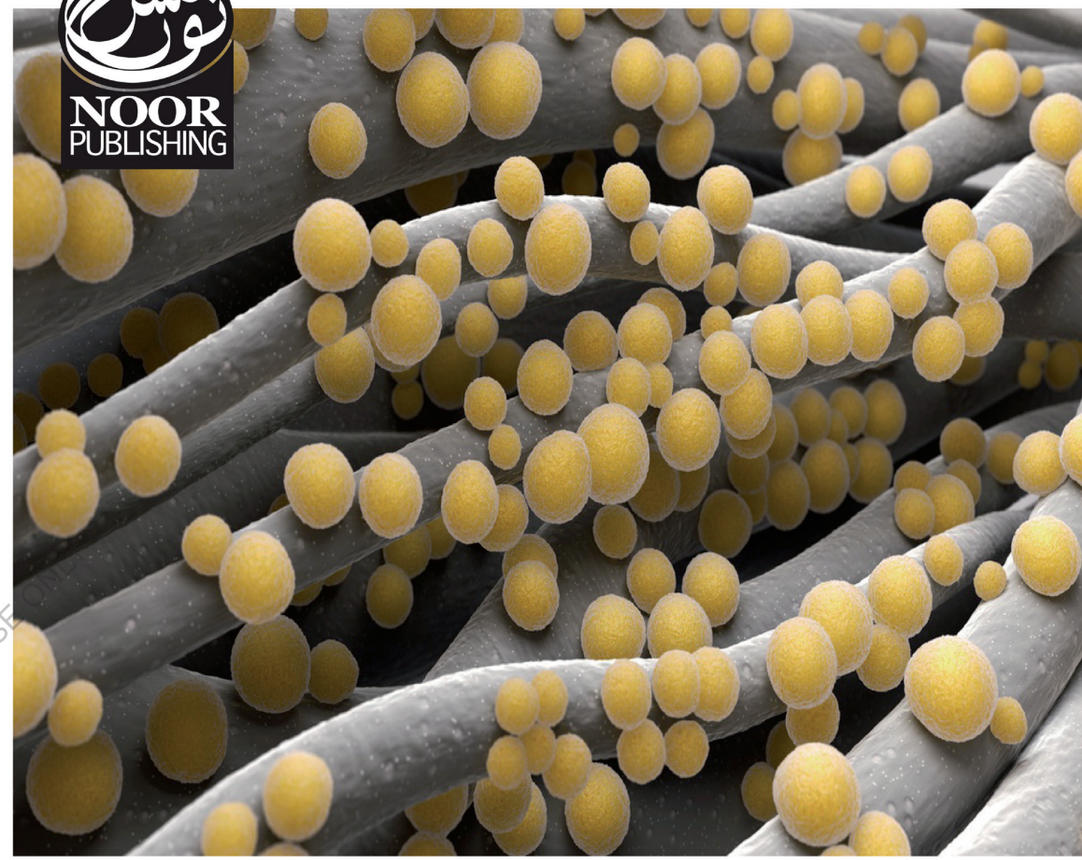

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ATOPIC DERMATITIS/ ECZEMA SYNDROME : A MOLECULAR INVESTIGATION OF MICROBIAL SUPERANTIGINICITY WIH BACTERIOLOGICAL , IMMUNOGICAL ,CLINICAL ,HEMATOLOGICAL AND HISTOPATHOLOGICAL STUDIES

BY

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DEDICATION

TO OUR LEADER TO WHOM ALL OF US ARE WAITING TO SEE (ALEMAM AL-MAHDI)

TO THE MEMORY OF MY FATHER
THAT ALWAYS REPRESENT WITH US

TO MY MOTHER , BROTHERS AND SISTERS.

TO MY DARLING WIFE & CHILDREN

WITH MY LOVE AND RESPECTION

I DEDICATE MY STUDY

IHSAN ALSAIMARY

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IHSAN ALSAIMARY

Summary

A multi-displenary study was carried on 484 patients suffering from atopic dermatitis (AD) (eczema) syndrome in Basrah city , 211(43.6%) males and 273(56.4%) females , with male: female ratio (1:1.29) , and a control group of 100 healthy individuals.

We can summarize the results as follow :

1- Clinical & epidemiological studies:

a- Infantile and childhood groups showed a higher prevalence of (34.7% and 27.5% respectively) than adult age group (37.9%). Males were highly affected with AD in age groups (1,4,5) , while females predominated male in groups(2,3) (P<0.001).

b- Eight locations of eczematous lesions were predominantly affected in patients with AD , face and face & neck were the commonest predilection sites of AD lesions (33.1% and 20% respectively) (P< 0.001).

c- Revealance of a high correlations between family and personal histories with AD(P< 0.001).

d- The skin in the majority of AD patients 289 (59.7% (109 M:180F)) was of dry type , followed by seborrheic and natural skin types (45M:78F and 57M:15F) respectively.

e- A subacute pattern of disease course and mild to severe degree of severity were found predominant in AD patients (P< 0.001)

2- Haematological study:

The values of Hb, RBCs count and basophiles were below the normal or control means in AD patients , while values of ESR, WBCs count , lymphocytes , eosinophiles and platelets counts were higher than normal means . On the other hand , the number of differential neutrophils , lymphocytes and monocytes were within normal means (P< 0.001) .

3- Immunological study:

a- The study showed a high significant elevation of various types of cluster of differentiation (CD): (CD3 ,CD4 , CD8 , CD19) .Also , all means of concentration of immunoglobulins (Ig) (IgA, IgG , IgM and IgE) and complement components C3 and C4 were increased more than those of control group($P<0.001$).

b- This study grouped AD patients into three groups of allergic modes according to the concentration of total IgE measured by (two- sites enzyme linked immunosorbent assay (ELISA), in which it was found that mode of allergy (very probable) (IgE> 100 IU) was predominant in 88,8% of various age groups ($P<0.001$)

c- Fourty environmental (food , fungal , agricultural , chemical and aero-) allergens , and eleven extracted bacterial antigens (as allergens) were tested against specific IgE by using EIA (enzyme immuno assay) technique, where we found a very highly significant allergens – specific IgE reaction with a high degree forms of hypersensitivity in AD patients ($P<0.001$) . We can arranged various allergens according to high degree of hypersensitivity as follows :

Sweet veneral grass>rose >sheep s wool >banana >codfish >cotton cultivated >common reed >scale >milk> chicken feathers >onion > cat epithelium >pigeon droppings >silk >chicken >pepper tree >plane tree >dog dander >yolk > cockroach > garlic > wheat > *Candida albicans* >olive > pencilloyl G> horse epithelium > egg white > cow dander >cheddar cheese

d- *Staph.aureus* exotoxin could be named as staphylogen of staphylogenic proteins recorded 100% for the highest degrees of hypersensitivity modes (B,A,H and more) . ($P<0.001$).

4- Bacteriological study :

a- The results revealed a positive cultures from the total AD patients (eczematous lesions and healthy skin) in 94.4% and 86.36% respectively in total number ranged from 0.02- 92.0 $x10^5$ cell/ cm and 0.11 – 23.0 $x10^3$ cell / cm in the same mentioned sites respectively .

b- *Staphylococcus aureus* was a predominant bacterial agent isolated in 60.48% and 17.48% from the above mentioned sites respectively, followed by other bacterial types in various percentages of isolation .

c- Antistaphylococcal activity of thirteen antibiotics were studied and revealed that Vancomycin is the best affective antibiotic according to antibiotics susceptibility test , followed by others.

d- A highly significant incidence of *Staph.aureus* resistance against double, three, four and five or more of antibiotics ($P<0.001$) .

5- ***Staph.aureus*** **exotoxin:**

a- A technique of five steps were used to isolate , purify , identify and characterize *Staph .aureus* exotoxin (staphylogen as a superantigen 0 includes: primary screening , primary detection, production assay , purification techniques, such as; precipitation by (NH4)2SO4 , membranous infiltration (dialysis) and gel filtration chromatography with Sephadex G-100 , and characterized the staphylogen by estimation of lytic activity , kinetic properties , cytotoxicity , antibacterial activity and evaluation of purity and molecular weight by Polyacrylamide gel electrophoresis(PAGE 7.5%) .

b- A highly purified single band protein of *Staph .aureus* exotoxin has a molecular weight of 9 47.315) Kd , and eight purified bands of all *Staph.aureus* antigens have a molecular weight ranged from (13.567 – 549.540)Kd.

6- ***In vivo*** **and histopathological studies :**

a- *In vivo* study successed in induction of eczema- like lesions on mice (BALB/C) skin that were experimenting infected with staphylogen , all bacterial antigens and two doses of viable cells and OMPs (outer membrane protein) of Staph.aureus ,by using a various infection methods : intradermal , spot and prick technique of injection .

b- Typical well known histopathological changes of eczematous lesions of AD patients were seen in our study and the same histopathological features were also shown in histological examination of slices from eczema- like lesions of mice skin.

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Chapter One
Introduction and Literatures Review

2. Introduction

1-1-1 - Atopic dermatitis: The concept

A definition of atopic dermatitis (AD) may be exceedingly simple: it is the cutaneous manifestation of atopy, well then, what is atopy? There is no doubt that the definition, this time, is not at all easy or simple. Etymologically, (atopy) means (without) (a) "place". (topos), that is, something that is strange, something that is unusual.(Gimenez, 2000 and Jordaan , 2005). Atopy as a term was introduced years ago to describe a group of patients who had a personal or family history of one or more of the following conditions: hay fever(allergic rhinitis), asthma, dry skin, and eczema. (Stanway,2005).Atopy is achieved not only through immunological mechanisms but through many other non immunological phenomena such as dryness of the skin, paradoxal skin reactivity, microorganisms, etc, which play a significant role in its development.(Kuster, *et al*.,1990 and Rothe, *et al*.,1996)

The manifestation of atopy may be quite varied, although Asthma, rhino-conjunctivitis and atopic dermatitis, the so-called "atopic triad" are the universally accepted ones. The inclusion of further clinical manifestations such as chronic urticaria, migraine, gastrointestinal conditions or epileptoid seizures is rather more controversial. (Comacho, 1998)

From the terminological point of view , the most adequate term for defining the process is "dermatitis", as an inflammation of the skin is in fact the underlying phenomenon of the disease.(Gimenez, 2000)

Atopic dermatitis (AD) is a chronic, Pruritic eczematous disease that nearly always begins in childhood and follows a remitting / flaring course that may continue throughout life.(Habif, 2004).

It develops as a result of a complex interrelationship of environmental, immunologic, genetic and pharmacologic factors. It may be exacerbated by infection, psychologic stress, seasonal / climate changes, irritants, and allergens. (Rystedt, 1985 and Cronin& Mcfadden,1993)

The disease often moderates with age, but patients carry a life-long skin sensitivity to irritants, and this atopy predisposes them to occupational skin diseases. (Hanifin & Chan, 1999).

The symptoms of atopic dermatitis are intense itching, erythema , and sensitivity of the skin that usually becomes worse at night and can disrupt sleep. The appearance and location of the rash may depend on age of the patient. In infants, atopy appears as a bubbling or oozing rash on the face, hand, and feet. In older children and adults a red, scaly, and itchy rash on the neck, hands, feet, and creases of the elbows and knees. During outbreaks, the rash may appear all over the body. (Correale, *et al*.,1999 ; Greene, 2002, and AAD, 2003a)

The most likely cause of AD is an overactive immune system. When a germ gets into your body, you produce an antibody that kills that germ. As soon as the germ is gone, your body stops making antibodies. In atopic dermatitis, the body keeps on making antibodies that end up in the skin to cause terrible itching. (Mirkin, 2003).

The exact pathogenesis of atopic dermatitis is unknown. Currently, it is believed that IGE- mediated reactions and cellular responses contribute to the chronic inflammation of this disorder. (Motala , 2004).

Atopic eczema typically runs a chronic course with exacerbations and remissions. A variety of "trigger factors" may exacerbate eczema: irritants (e.g. soap, harsh chemicals), heat and humidity, stress and anxiety, certain foods, inhalant allergens and certain infections. Egg, milk, wheat, Soya protein and peanut are the most common offending foods. Inhalant allergens such as house, dust mites , pets, pollens and cut grass may also cause a cute flare-up of eczema. (Beers & Berkow , 2003 and NIAMS, 2003a)

Patients with atopic dermatitis are highly susceptible to secondary bacterial infections (e.g.staphylococcal and streptococcal) and viral infections, (e.g. warts, Molluscum contagiosum and Herpes simplex) and occasionally to fungal infections (e.g. *Pitysporrum ovale*). (Giordanolabadie, 1998 ; Correale, *etal.,* 1999 and Mirkin, 2003)

People who have atopic dermatitis are particularly prone to skin infection. This is in part due to the breaks in the skin from very dry, split skin and from scratching the itchy areas.(Stanway, 2004).

People with atopic dermatitis also seem to have a reduced ability to fight against these common bacteria on the skin. As a result people with atopic dermatitis frequently suffer from boils, folliculitis and inflections of their eczema. This begins vicious cycle as infection cause the eczema to worsen and become more resistant to the usual treatment with emollients and topical steroids. Antibodies are often required to eliminate the inflection before the eczema can once be more brought under control. (Saurat & Hanifin, 2001 ; Leung, *etal* ., 2004 and Stanway, 2004;).

Staphylococcal and streptococcal infections are common complications of atopic lesions. Heightened susceptibility to infections occur because the skin of patients with atopic dermatitis is a poor barrier against organisms, has depressed immune function and lacks normal lipophilic bacteria.(Rystedt, *etal* ., 1989 ;Correale, *etal* ., 1999 and Antonio , *etal* ., 2004)

1-1-2- Some evidences of atopic dermatitis

- Atopic dermatitis is very common. It affects males and females equally, and counts for 10 to 20 percent of all visits of dermatologists. (NIAMS,2003a and Motala, 2004)
- Up to 15% of population may suffer from atopic dermatitis during childhood. (Mirkin, 2003 and Leung , *etal* ., 2004)
- More than 15 million people in the United States have symptoms of atopic dermatitis occurs in 5-25 per 1000 people. (Fewell , 2003)

- Although atopic dermatitis may occur at any age, it most often begins in infancy and childhood.(NIAMS , 2002)
- 65% of patients develop symptoms in the first year of life, and 90% develop symptoms before the age of 5. (Lapidus ,*etal* ., 1993)
- Onset after age 30 is less common and is often due to exposure of the skin to harsh or wet conditions.(Linde , 1992 ; Leung, 1995, and Mirkin , 2003)

- Atopic dermatitis is a common causes of workplace disability. (Adinoff , *etal* ., 1988 and Leung, *etal* ., 2004)
- People who live in cities and in dry climates appear more likely to develop this condition. (Fewell, 2003)
- 20% of infants and young childhood experience symptoms of the disease. Roughly 60% of these infants continue to have one or more symptoms of atopic dermatitis in adult hood. (NIAMS , 2003b)
- There are about 100 – 1000 articles and / or researches interested in atopic dermatitis (eczema) published monthly in various internet loci .

1-1-3- Aims of the study

To the best of our knowledge , there are no published scientific studies about atopic dermatitis neither in Iraq nor in Basrah community , So, the present study aimed to:

1. Determine the clinical and epidemiological markers of this disease in various age groups and in both sexes.
2. Isolate and identify the major bacterial types predominate in eczematous lesions and healthy skin of patients with atopic dermatitis, with estimation of antibiotics susceptibility of main common bacterial types.
3. Isolate and purify the toxins and / or enzymes that are produced by main common bacterial types isolated from eczematous lesions.
4. Apply *In Vivo* study to reveal the effects of these toxins/ enzymes on BALB/c mice
5. Apply immunological study for all important immunologic markers in patient sera associated with atopic dermatitis including : Immunoglobulins; IgM, IgA and IgG. Complement; C3 and C4 , total IgE and specific IgE against most important allergens and cluster of designation (differentiation); CD3, CD4, CD8 and CD19
6. Apply haematological study of AD patients blood components .
7. Apply histopathological studies on human patients with atopic dermatitis and experimentally induced animal (BALB/C Mice) lesions to determine the main histopathological changes in induced mice skin lesions (of epidermis and dermis) in comparison with that of human – in especially- to find out if there are any similarities between eczematous lesions of human and mice.

2. Review of Literatures

1-2-1 - Historical aspects

Willan is credited with the first descriptions of itchy conditions in 1808. In 1884 VonHebra postulated that a papule preceded the itch of AD. The contrary view was recognized and reported in 1904 by Jacquet. His statement that "this is an itch that erupts and not an eruption that itches" has subsequently become the most frequently quoted description of AD, (Despite not being totally correct- it is an itch that when scratched erupts). Besnier noted the association of this itchy skin disease with asthma, hay fever and gastrointestinal disturbances in 1892. He also suggested that the disease tended to be familial and to occur in those constitutionally predisposed . (Beltrani, 1996; Pakster & Parnir, 2002 and Stanway, 2005)

In 1923 Coca and Cooke introduced the term ((atopy)) to designate the phenomenon of hypersensitivity in human. Atopy was adapted from the Greek word meaning "out of place" or "strange disease". (Wuthrich & Grendelmeier , 2002).

The term "atopic dermatitis" was first proposed by Wise & Sulzberger in the 1933, because of the close association with other atopic diseases such as bronchial asthma and allergic rhinitis (Grendelmeier, etal; 2001)

In 1959, Schnyder reported that the incidence of allergic rhinitis was ten times and asthma five times more common than AD. (Beltrani, 1996). Many population studies, particularly those conducted by Schwartz,(1952) and by Schnyder,(1960), and follow-up studies of children first seen with infantile eczema, supported the close association of bronchial asthma, hay fever perennial rhinitis, atopic dermatitis, and some forms of food allergies with classical atopic disease (AAD, 2000a and Wollenberg & Bieber, 2000).

Ishizaka and Ishizaka in (1967) and Johansson in (1967) identified a new class of immunoglobulins , the IgE antibodies, as a carrier of reaginic activity, raised in atopic individuals, and, therefore, a characteristic of the atopic condition. Shortly after the discovery of the IgE molecule it was shown that serum IgE level in atopic dermatitis are increased an average, and that this serum IgE increase in relation to the increase of specific IgE against several environmental allergens. (Wuthrich & Grendelmeier, 2002).

In 1976, Spector and Fair discussed the different immunological and pharamacological characteristics of atopic individuals and of asthmatic subjects, such as elevated IgE levels, impaired T-cell function, decreased B-adrenergic receptor responsiveness, etc.(Williams, 1999).

In 1979 Lowell proposed redefining atopy to include the respiratory syndromes of asthma and rhinitis, allergic (IgE- mediated) or nonallergic, which share a genetic predisposition, esinophilia, hyperreactivity to an x- adrenergic agent, and responsiveness to steroid. However, this author, like coca and cooke, had omitted to include atopic dermatitis in the definition of atopy. (Mygind, etal; 1996).

1-2-2 - The concept and definition of atopy and atopic dermatitis

Atopy was defined by Coca and Cooke, (1923) as "genetically determined abnormal state of hypersensitivity as distinguished from hypersensitivity in normal individuals, which is not genetically determined". Atopy in the concept of Coca and Cooke is : (1) hereditary, (2) limited to small group of human beings, (3) different from anaphylaxis, referring to a

lack of protection, and allergy, meaning an altered reactivity, both of which can also be induced experimentally in humans and in animals, (4) qualitatively an abnormal response occurring only in particular individuals (atopic), (5) clinically characterized by hay fever and bronchial asthma, and (6) associated with immediate- type (flare-and-wheal) skin reactions. (Wuthrich & Grendelmeier, 2002).

Wise and Sulzberger discussed in the (1933 year book of Dermatology and Syphilogy) the conditions of eczema, neurodermatitis, lichenifecation and "Prurigo diatheśiques" described by the French dermatologists, Besnier and Brocq stated in a footnote that: of all of these forms of more or less confused and confusing types of localized and generalized lichenifecation at least one is emerging as a fairly distinct and clear-cut entity. This is probably best called "atopic dermatitis", but is generally known as generalized neurodermatitis or diffuse pruritus with lichenifecation. This is characterized by the following cardinal qualities:- (1) atopic family history, (2) antecedent infantile eczema, (3) localization in antecubital and popliteal spaces, the anterior portion of the neck and chest and the face, particularly the eye-lids, (4) the presence of a grayish or brownish coloration of the skin, (5) the absence of true vesicles, clinically and histologically, (6) vasomotor instability or irritability, (7) the usual negative patch test with many contact allergens, (8) many positive reaction of immediate wheal type to scratch or intradermal testing and (a) the presence of many regions in the blood serum. (Fabrizi, *etal.* , 1999 ; Wuthrich, 1999, and Wuthrich & Grendelmeier, 2002).

The varied clinical presentation and suspected etiologies have resulted in many labels for the same affliction, including: eczema constitutionnel, eczema infantum (in infancy), eczema flexurarum (in childhood), prurigo diastheque, prurigo Besnier, Neurodermatitis constitutionals, neurodermatitis disseminate and pruriginosa (while and after puberty), endogens Eczema, fruh-und spatexsudative Eczematoid , Asthma- eczema, diffuse neurodermatitis, food eczema, infantile eczema, status neurodermiticus, intrinsic allergic dermatitis, and atopic dermatitis. (Beltrani, 1996)

There are many various definitions of atopic dermatitis in recent literatures as follow: AD is a common inflammatory skin disease characterized by severe pruritus, a chronically relapsing course which often starts during infancy, a distinctive clinical appearance, e.g., flexural involvement, and several clinical, immunologic, and biochemical alteration.(Cooper, 1994; leung, 1995, and Wuthrich, 1999) . AD is an inflammatory skin disease, characterized by an itchy, erythmatous, poorly demarcated skin eruption, which has a predilection for the skin creases. (Charman, 1999) .AD is a chronic pruritic skin condition usually begins in infancy. (Ghidorzi, 2001) . AD is a chronic, itching, inflammatory skin disease associated with asthma and/ or hay fever and a familial occurrence of these conditions. (Faergemann, 2002). AD is a chronically relapsing skin disorder with an, immunologic basis. The clinical presentation varies from mild to vary sever and, in the worst cases, may interferes with normal growth and development. (Spangola & Korb, 2002). AD is a chronic (long-lasting) disease that affects the skin. It is not contagious, it cannot de passed from one person to another, it is often an inherited tendency to develop other allergic conditions. (NIAMS, 2003a). AD is a common chronic inflammatory skin disorder that is regarded as a typical multifactorial disease . The etiology is complex and the disease is caused by concerted actions of environmental and genetic factors . (Kluken , *et al* ., 2003) . AD is excess inflammation in the skin , linings of the nose , and lung . It often runs in families . (AAD, 2003) .AD is a chronic, pruritic, superficial inflammation of the skin, frequently associated with a personal or family history of allergic disorders (e.g., hay fever, asthma). (Beers & Berkow, 2003) . AD is a well-described skin condition that may include other systemic manifestations such as asthma, rhinitis and seasonal allergies. (Ming, etal; 2004) and AD is a chronic inflammatory skin

disease associated with cutaneous hyperreactivity to environmental triggers and is often the first step in the atopic march that results in asthma and allergic rhinitis. (leung, etal; 2004). In the recently proposed nomenclature by the European Academy of Allergology and Clinical Immunology, AD has been renamed as atopic eczema/ dermatitis syndrome (AEDS), and divided into: non allergic and allergic AEDS, the latter being subdivided into "IgE-associated AEDS" and "non- IgE-associated allergic AEDS". (Wuthrich & Grendelmeier, 2003).

1-2-3- Eczema and Atopic dermatitis

Eczema is a general term encompassing various inflamed skin conditions. One of the most common forms of eczema is atopic dermatitis (or atopic eczema). Approximately 10 % - 20 % of the world population is affected by this chronic, relapsing, and very itchy rash at some point during childhood. Fortunately, many children with eczema find that the disease clears and often disappears with age. (AAD.2000b)

In general, atopic dermatitis will come and go, often based on external factor. Although its cause is unknown, the condition appears to be an abnormal response of the body's immune system. (AAD.2003).

In people with eczema, the inflammatory response to irritating substances is overactive, causing, itching and scratching. Eczema like many diseases, currently cannot be cured. However, for most patients the condition may be managed well with treatment and avoidance of triggers. (NIAMS, 2002).

1-2-4- Type of Eczema (Dermatitis)

1. Allergic contact eczema (dermatitis): a red, itchy, weepy reaction where the skin has come into contact with a substance that the immune system recognizes as foreign, such as poison or certain preservatives in creams and lotions .
2. Atopic dermatitis: a chronic skin disease characterized by itchy, inflamed skin.
3. Contact eczema: a localized reaction that includes redness, itching, and burning where the skin has come into contact with an allergen (an allergy-causing substance) or with an irritant such as an acid, a cleaning agent, or other chemical.
4. Dyshidrotic eczema: irritation of the skin on the palms of hands and soles of the feet characterized by clear, deep blisters that itch and burn.
5. Neurodermatitis: scaly patches of the skin on the head, lower legs, generally related to circulatory problems.

(NIAMS, 2003, a&b).

1-2-5- Diagnostic Criteria of atopic dermatitis

The most important diagnostic criteria for clinical diagnosis of atopic dermatitis are:

1. Hanifin and Rajka criteria in (1980) and largely adopted by the American Academy of Allergy, Asthma, and Immunology. Appropriate cases must have at least three major characteristics and at least three minor characteristics. (Hanifin & Rajka, 1980)
2. The Lillehammer criteria in (1994) are based on the idea that the distribution of the atopic dermatitis may differ in the infantile, childhood, and adult phases. (Larsen, etal; 1996).

3. Diagnostic criteria of United Kingdom working party (Diagnostic scorecards) in 1994 . It is a simplified way for physicians to make the diagnosis. It requires a pruritic skin condition and three additional criteria, which may include the followings:

a- History of flexural dermatitis or dermatitis on the cheeks in children less than 10 years of age.

b- A personal history of asthma or hay fever or atopy in a first-degree relative if the patient is less than 4 years of age.

c- A generalized xerosis in the last year.

d- A visible flexural eczema or eczema on the Cheek or forehead, or both, and on the extensor extremities if the patient is less than 4 years of age. (Wuthrich & Grendelmeier, 2002).

4- Spergel and Schneider criteria (1999) proposed that the diagnostic of atopic dermatitis has to be made by constellation of physical findings. The major features include: Pruritis, typical morphology and distribution of the lesions. The skin distribution varies with age. In infancy, the face and extensor surfaces of the arms and leg are most commonly affected. (Spergel & Schneider, 1999).

Other diagnostic criteria were elaborated in Germany by Diepgen, *etal.* (1989), and in the United Kingdom by Williams, etal; (1996) and Williams, (1999).

The modern latest criteria was proposed by Williams , (2005) depend on presence of sleep disturbance , the number and location of involved sites and the clinical course that indicators of severity .

1-2-6- Clinical features of atopic dermatitis figure (1)

1-2-6-1- Itching, the primary lesion:

Atopic dermatitis starts with itching. Abnormally dry skin and a lowered threshold for itchy are important features of AD. It is the scratching that creates most of the characteristic patterns of the disease. Most patients with AD make a determined effort to control their scratching, but during sleep conscious control is lost; under warm cover the patient scratches and a rash a pear (Weedon , 2002). The itch-scratch cycle is established, and conscious effort is no longer sufficient to control scratching. The act of scratching becomes habitual, and the disease progresses. Atopic skin is associated with a lowered threshold of responsiveness to irritants. (Ghidorzi, 2001, Beltrani & Boguneiwicz, 2003, and Habif, 2004). The quality of pruritus is referred to as a spreading itch. (Spagnola & Korb, 2002).

1-2-6-2- Other clinical finding

The rash of atopic dermatitis is not in itself particularly distinctive. It consists of erythmatous , elevated, scaly, and often excoriated and oozing plagues. (Leicht & Hanggi, 2001)

The plagues show Lichenification, or a thickening of the skin with exaggeration of skin lines, considered to be atrophic response to chronic rubbing.(Morren, etal, 1994)

Scratching and rubbing universally act as a trigger, that cause flare, spread of the skin disease, and account for popular eruption.(Roth & Grant, 1996)

The affected skin is dry, rough, and lusterless, and no amount of moisturizer seems to be effective. Usually, no gross scaling is visible on clinically uninflamed skin unless a hereditary ichthyosis is also present. (Morren, etal; 1994).

The dryness is due to the underlying mild, generalized cutaneous inflammation that alters the normal barrier function of skin and disturbs the structure and differentiation of the stratum corneum of the epidermidis. This xerosis improves when the inflammatory process is controlled. (Ely, 1997; Weedon , 2002 and Beltrani & Boguneiwicz, 2003,).

Atopic dermatitis usually begins as dry, scaly, cracked, erythematous patches that appear bilaterally on the cheeks. It may extend to the rest of the face, to the neck, wrists, and hands, and to dorsal aspects of the upper extremities. (Halbert, *etal*., 1995; Hanifin & chan, 1999, and Leicht & Hanggi; 2001).

1-2-7- Atopic dermatitis phases

AD is arbitrarily divided into three phases :

1-2-7-1- Infant (Infantile) phase (birth to 2 years)

Infants are rarely born with atopic eczema, but they typically develop the first signs of inflammation during the third month of life. (Habif, 2004).

The most common occurrence is that of a baby who during the winter months develop dry, red, scaly areas confined to the cheeks, but sparing the perioral and paranasal areas. (AAD, 2003).

This is the same area that becomes flushed wit exposure to cold. The chin is often involved and initially may be more inflamed than the cheeks because of the irritation of drooling and subsequent repeated washing. Inflammation may spread to involve the paranasal and perioral area as the winter progresses. Habitual lip licking by an atopic child results in oozing, crushing, and scaling on the lips and perioral skin. (Charman, 1999; Mirkin, 2003; and Habif, 2004).

The course of the disease may be influenced by events such as teething, respiratory, respiratory inflections, and adverse emotional stimuli. The disease is chronic, with periods of exacerbation and remission, and resolves in approximately 50% of infants by 18 months; other cases progress to the childhood phases and a different pattern evolves. (Green, 2002; Eichenfield, *etal*., 2003, and Stanway, 2004).

1-2-7-2- Childhood phase (2 to 12 years)

The most common and characteristic appearance of AD is inflammation in flexural areas (i.e., the antecubital fossae, wrists, and ankles. (Habif, 2004)

The eruption begins with papules that rapidly coalesce into plaques, which become lichenified when scratched. (Barnetson & Rogers, 2002)

The exudative lesions typically of the infant phase are not as common. Most patients with chronic lesions tolerate their disease and sleep well. (David, 1991 and Tofte & Hanifin, 2001).

There are probably several reasons for increased incidence of atopic dermatitis among children in developed countries, including high exposure to air pollution, smaller families with exposure to infections, more pets, higher maternal age, and a winder rang of food. There is clearly also an important hereditary component to atopic dermatitis (atopic eczema). (Eigenmann, *etal*., 1998; Itowlett, 1999 and Murphy & Atherton, 2001).

Recent studies discovered that standing in a hot shower give considerable temporary relief, but further progression is inevitable with the drying effect produced by repeated wetting and drying. In the more advanced cases, hospitalization is required. Most patients with this pattern of inflammation are in remission by age 30, but in a few patients the disease becomes chronic or improves only to relapse during a change of season or at some other period of transition. The dermatitis becomes a lifelong or deal. (NIAMS, 2003a and Habif, 2004).

1-2-7-3- Adult phase (12 years to adult)

The adult phase of AD begins near the onset of puberty. The reason for resurgence of inflammation at this time is not understood. But it may be related to hormonal changes or to the stress of early adolescence. Adults may have no history of dermatitis in earlier years, but this is unusual. (Habif, 2004)

As in the childhood phase, localized inflammation with lichenifecation is the most common pattern. One or several areas may be involved, and there are several characteristic patterns. (Hanifin & Chan, 1999 ; NIAMS, 2003b).

A Norwegian study based on review of medical records found a prevalence of atopic dermatitis of 13% in patients under the age of 20 years compared to 2% for those the age of 20 years. (Falk, 1993).

In the United Kingdom 2% of adult aged 16-40 years and less than 0.2% of adults over the age of 40 years are affected. (Herd, *etal*., 1996). Adult atopic dermatitis has been relatively ignored in epidemiological research, however, in developed countries adults make up approximately 80% of the population, meaning that up to a third of all subjects with disease are adults. (Charman & Williams, 2002).

Furthermore, there is evidence that the number of adults presenting to hospital with the disease is increasing in some countries. (Nishioka, 1996).

1-2-8- The skin

The skin is our principle organ of beauty, touch, pleasure, and sensuality. The skin is the largest organ of the body, accounting for 12%-16% body weight cover 12 to 20 square feet. With age the amount of subcutaneous (under-the-skin) fat is reduced resulting in a looser look to the skin. (Murphy, 1997).

The approximate chemical composition of the skin is :water 70%, protein 25.5%, lipids 2.0%, Trace minerals 0.5%, and rest 2.0%. (Pickart, 2005).

The structure of Human skin

The skin is composed of three main layers: **figure(2)**

1-2-8-1- Epidermis

The epidermis is purely a cellular structure without blood vessels. The different layers of the epidermis include: the stratum corneum, stratum granulosum , stratum malpighii (prickle cell layer) , stratum spinosum (ret mucosum) and the basal layer (stratum germinativum) . In palmoplanter skin there is an additional zone , the stratum lucidum between the stratum granulosum and the stratum corneum . (Pickart, 2005)

The epidermis is maintained by the division of the germinate cells presenting the basal layer and differentiate later Keratinocytes in about seven weeks (Murphy, 1997).

There is a thin film of sebum covering the top layer of the epidermis (the stratum corneum). This is a waxy mixture of lipids and cell fragments secreted by sebaceous glands, which also contains a mixture of bacteria that do not normally invade the body any further. Sebum helps to keep the skin moist and stratum corneum healthy. The epidermis doesn't contain blood vessels, but has nerve ending and occasional muscle fibers. (Heynen, 2004).

Epidermis cells are composed of keratinocytes, melonocytes, mast, langerhans cells and undefined cell.

1-2-8-1-1-Keratinocytes

As the keratinocytes moves upwards reaching the granular layer of the epidermis, loose their nuclei. Keratinocytes become flattened and heaped on the skin surface as horny dead cell. The thickness of this layer varies according to age and different body sites. (Murphy, 1997)

Vitamin A is found to have an important role in the development of keratinocytes. (Pickart, 2005)

Keratinocytes possess A and B blood group antigens and share with the red blood cells the same antibodies that are absorbed selectively in some immune reactions. (Bos & Kapsenberg, 1993)

The functions of Keratinocytes are: (Amagi, 1995 and Pickart, 2005)

1. Synthesis of fibrillar proteins giving the stratum corneum its toughness.
2. Secretion of a large number of cytokines, which have an important effect on lymphocyte and granulocyte functions.
3. Synthesis of a wide range of growth factors that have an important role in wound healing. The factors controlling synthesis and secretion of these factors may be important in the pathogenesis of skin disease.
4. give a partial protection from U.V. radiation damage

1-2-8-1-2- Langerhans cells

Have immunological functions, which can provide traps for contact antigens and present them to T- cells. (Katz, 1993).

1-2-8-1-3- Melanocytes

Are dendritic cells derived from the neural crest and lie on the basement membrane. Melanocytes synthesize melanin from phenylalanine via tyrosine by series of reactions catalyzed initially by tyrosinase giving the skin its color. Pigmentation is related more to melanin synthesis than to the number of skin melanocytes. (Valyi, *etal*., 1990)

1-2-8-2- Dermis (corium)

the dermis contains connective tissue, blood vessels, lymph vessels, occasional white blood cells(lymphocytes and other cells concerned with immunity), never endings , hair follicles, sweat glands and sebaceous glands and muscle elements. (Heynen, 2004)

The cells of dermis are deriven from the reticulum cell, the primitive mesenchymal cell. The cells of the dermis include:

1. **fibroblasts**: that are responsible for :
a. The manufacture of collagen and elastic fibers, which provide the skin with its toughness and elasticity.
b. Synthesize the mucopolysaccharides, and
c. Metabolize cholesterol and steroids. (Pickart, 2005)

2. **Histiocytes** : Are part of the reticuloendothelial system. Histiocytes are large phagocytic cells either fixed to the interstitial tissues or wandering. (Shimada & Katz, 1988)
3. **Mastocytes**: Are specialized cells that synthesize histamine and heparin. Mast cells are numerous in the capillary layer of the epidermis, in the beds of capillaries, in the reticular layer and in the areolar tissue that surrounds the cutaneous appendages. (Sueki, etal; 1993)
4. **Lymphocytes**: Have an important role in the immune reactions. (Katz, 1993)
5. **Plasma cells**: From immunoglobulins (Ig) antibodies. (Pickart, 2005)
6. **Eosinophils**: Inhibit the action of histamine (Bos& Kapsenberg, 1993).

1-2-8-3-The Hypodermis (subcutis, subcutaneous layer or subcutaneous adipose tissue)

The subcutis is the deepest later of the skin, composed primarily of fat. The subcutaneous layer manage the skin's function of feeding, excreting and heat exchange. The key cells are fat cells or adipocytes that provide energy, serve as a heat insulator for the body, and act as a shock absorber to protect underlying tissues against mechanical trauma and give the skin its resilience. Among mammals, only humans and marine mammals such as whales and dolphins have this subcutaneous layer of fat. Sweet glands originate in this layer and excrete waste matter through perspiration. This sweat controls the body's temperature by evaporating and cooling the skin surface. (Pickart, 2005)

1-2-9- Histopathological features of atopic dermatitis

In atopic dermatitis, where the epidermis is under the influence of increased production of cytokines and other local growth factors and inflammatory substances, the keratinization process is accelerated , the skin becomes thicker and drier, and visible scaling may occur. (Leung, 2000)

These substances also stimulate the nerve ending, sending an unpleasant itching sensation to the brain, which results in scratching (Leung, 1995).The mechanical effect of scratching is itself a powerful signal for keratinocytes to produce more local cytokines, starting the infamous itch-scratch vicious cycle. Worse, the scratch disrupts the physical barrier of the close-packed cells, allowing bacteria to invade the epidermis, causing additional inflammation (Leung, 1992 and Heynen, 2004)

The **figure (3)** represents changes that occur in skin affected by atopic dermatitis as follows (Heynen, 2004).
1. **Intracellular edema (Spongiosis)** : Fluid accumulates in the epidermis as cells move out of the blood vessels and into the skin, during the inflammation of dermatitis.
2. **Acanthosis**: Is a variable thickening of the cells of stratum spinosum.
3. **Hyperplasia**: An increase in cells of the stratum corneum, in response to local tissue damage by inflammation or scratching, which is more than sebum production can scope

with the skin becomes dry (Xerosis), thick and in elastic (this skin is said to lichenified).It is subject to painful fissures.

4. **Papule**: A small, visible, palpable, flat-topped, firm lesion, raised above the skin surface (a papule larger than 1 cm diameter is called a plaque).
5. **Vesicle**: A small blister (less than 1 cm diameter). Larger blisters, called bullae, are more likely to occur in irritant contact dermatitis than allergic contact dermatitis or atopic dermatitis.
6. **Erythema** : Is reddened skin.
7. **Scaling** becomes excessive and visible.
8. **Weeping lesions** are scratched and ruptured lesions, which ooze serum, dried blood, scales and pus.
9. **Crust dried matter** from a weeping lesion, yellow in color.
10. **Excoriations**: Scratch marks on the skin's surface. In patients with chronic dermatitis, excoriations are clearly visible on the skin some time after the person has scratched themselves.
11. **Fissure**: A linear crack which extends at least into the dermis of excessively dry skin. occurs in chronic dermatitis.
12. **Pain receptors stimulated-itch** :Is a sensation that only occurs in skin and it is a major feature of dermatitis.
13. **Cells migrating** from the blood vessels of the dermis.
14. **Vesicle forming**.

1-2-10- Bacteriology of Atopic dermatitis

1-2-10-1- Bacterial Pathogenesis

Robert Koch, in the late of nineteenth century described a number of requirements that must be satisfied before an organism can definitely be regarded as the cause of the disease. These condition have become known as "Koch's postulates or Germ Theory of Disease" in 1884, and they can be summarized as follows: (DWorkin, 2002)

1. **Microorganism must be present in all cases of the disease and absent from healthy individuals.**
2. **Organism must be isolated and grown in pure culture.**
3. **Upon infection of a healthy animal with the pure culture, the same disease must be experimentally produced.**
4. **The same organism must be reisolated from the experimentally infected animal**.

We now understand that disease is a far more complex interplay of the organism's pathogenic mechanisms, the host defenses and environmental stresses.(DWorkin, 2002).

1-2-10-2- The bacterial skin flora

1-2-10-2-1- In healthy non atopics

The skin becomes colonized by normal flora from birth. Colonization of the skin by normal skin flora is related to different factors:

1. **Age**: In infants and children micrococci are more prominent than in adult's skin. In adults *Propionibacterium* are more due to the increased skin lipids. In old age streptococci and enterococci become residents of skin especially in moist areas(Habif, 2004)
2. **Sex**: Males carry higher numbers of bacteria than females.(Rozsypal,2004)

3. **Race**: Negroes have less nasal carriage of staphylococci than Caucasians.(Rozsypal,2004)
4. **Type of colonizing strains**: Colonization of one area of skin by one strain of staphylococci interferes with colonization by another species.(Antonio, *etal* ., 2004)
5. **Skin conditions**: The skin provides a dry, mechanical barrier from which contaminating organisms on the surface are constantly removed by desquamation. Resident bacterial skin flora are found more on moist areas containing abundant sebaceous glands, while dry skin has less resident normal flora(Habif, 2004) . Huge number of harmless normal bacterial flora colonization the normal skin. These may be resident on the skin and its appendages or transient flora. When the immune condition is impaired or the skin is irritated or injured, non- pathogenic organisms may change their behavior and become pathogenic. (Rozsypal, 2004).

The mechanism of pathogenicity and even the same strain virulence and ability to cause inflammation depend mainly on :
1. The states of the skin epithelium and its secretions.
2. The cellular and humoral factors
3. Interaction between the commensal organisms and other organisms.
4. Permanent eradication of carriage of *Staph.aureus* is not possible, but temporary elimination may be by oral or tropical antibiotics but soon there will be recolonization after stopping the antibiotics. (DWorkin, 2002 and Habif, 2004)

The normal skin has probably a defense mechanism against bacterial infection, *Propionibacterium acnes, P. granulosum* and Gram positive cocci may cause split of fatty acid into free fatty acid and triglycerides, which cause skin irritation and inflammation as the case in acne. (Hauser, *etal*., 1996).

Some strain of normal skin flora can produce antibiotics capable of inhibiting other microorganisms. (DWorkin, 2002).

Bacteria that are consistently cultured from the skin; that are relatively stable in number and that can multiply on normal skin have been termed residents. A second group of bacteria belong to the transient flora ; they comprise a wider variety of bacterial species and may be derived from exogenous sources or carried over from the mucous membranes or the intestinal tract.(Roth &James, 1989).Some authors have defined a third group of temporary residents which may occasionally colonize the skin of some individual under certain environmental conditions. (Hauser, *etal*.,1996).

The resident bacterial flora is located in the outermost layer of the epidermis. Small bacterial colonies have been identified at the skin surface. There is a wide regional variability in the composition and the number of bacteria found. (Roth & James, 1989). However, differences between individuals at the same body location are relatively small. Thus a sample from a single body site cannot be regarded as representative for the entire skin flora. (Hauser, etal; 1996).

It is generally accepted that the normal resident skin flora includes: Micrococci, Staphylococci (e.g. *Staph.epidermidis* and *Staph.capitis*), Corynebacteria (e.g. *C. minutissimum*), propionibacteria (e.g. *Pr. acnes* and *Pr. granulosum*), and *Acetinobacter*. Anaerobes outnumber aerobes by a factor more than 10, the latter containing most of the pathogenic organisms. (Tuazon, 1984; mesenga, *etal*., 1990, and Hauser, *etal*., 1996)

1-2-10-2-2- In patients with atopic dermatitis

The skin of patients with AD is characterized by higher levels of colonization by aerobes. Significantly higher colony forming units per surface unit(CFU) could be isolated

from clinically normal skin of patients with AD than from corresponding skin sites in normal control. (Hauser, *etal*., 1985 and Ogawa, *etal*., 1994). The difference being 1 to 2 orders of magnitude. The highest counts, however have been made in samples from lesional skin. Acute exudative lesions yielded a mean of 10^7 colony forming unites (CFU) $Icm^{2.}$ Thus the atopic skin must provide a favorable environment for the colonization and multiplication of aerobic bacteria (Watanabe, *etal*., 2003 and Antonio, *etal* ., 2004).

Coagulase positive staphylococci (COPS)(*Staph.aureus*), which are usually not found on normal skin accounted for the majority of CFU isolated from AD skin. But the number of coagulase - negative staphylococci (CONS) was also increased. (Hauser, *etal*., 1996). Although coagulase – negative staphylococci have generally been regarded as being of low virulence, the role of coagulase – negative staphylococci as pathogens is becoming increasingly recognized. (Leyden, *etal*., 1993 and Antonio, *etal* ., 2004)

1-2-10-3- Bacterial Superantigens

Superantigens are high molecular weight protein comprising a group of molecules produced by various microorganisms, such us bacteria (Staphylococci, Streptococci, *Yersinia* , and *Mycoplasma*) and viruses (Narat , 1998). They are involved in the pathogenesis of several human diseases including food poisoning, septic shock, Kawasaki syndrome, and psoriasis (Schlievert, 1993). Bacterial superantigens can active T cells in an MHC-unrestricted, unspecific way by interacting directly with the TCR-Vβ chain and the MHC class II molecule outside the peptide-binding groove area (Fleischer, *etal*., 1991; Kappler, *etal*., 1992; Mollick, 1993 and Torres , *etal*.,2001). The toxins have significant binding affinities for certain defined constant parts of the TCR-VB-chain (about 2-6 per toxin). Both autologous and allogeneic, and even xenogeneic, antigen-presenting cells (APC) are able to stimulate up to 25% of the T-cell pool in the presence of superantigens, which are strong mitogens even in a concentration of 10^{-10} M (Carlsson, *etal*., 1988). On the other hand a " conventional" allergen which undergoes classical antigen presentation may stimulate about 0.1% of allergen-specific T cells in a MHC- restricted fashion via their TCR-Vα and β chain . **Fig(4)**

The influence of bacterial superantigens on the immune system has been well investigated particularly in animal models (Breuer, *etal*., 2001). After intravenous application of superantigen (1μg of SEB) in mice, almost all V β8+ T cells become activated, leading to a substantial production of cytokines and a loss of L- selection on the cell surface. In the initial phase of T- cell activation 1-4 hr after application of superantigens, TNF-α , IL-2, IL-4, IL-6 and IFN-Y are traceable in the murine serum. This initial phase is followed by a (partial) deletion and anergy of SEB-reactive cells (Miethke, *etal*., 1995). T- cells activated by superantigens undergo apoptosis after a distinct number of cell divisions (Renno, *etal*., 1999).

1-2-10-4- Skin Colonization by *Staph.aureus*

Staph. aureus may be isolated from the skin of healthy individuals. (Hauser, *etal*., 1996). But it is not considered to be a member of the normal resident flora. (Williams & Mackie, 1993).In a large study of 382 healthy children and 378 normal adults, *Staph. aureus* was recovered from < 5% of the skin isolates. low rates of *Staph. aureus* positive skin samples were also reported in several other studies (Hauser, *etal*., 1985; Barth, 1987; and Hauser, *etal*., 1996).

Nasal carriage, reported to be up to 40%, is more frequent than cutaneous colonization of healthy subjects. (Barth, 1987). Chronic nasal carriers have an increased risk of skin inflections (impetigo and furunculosis) and of post operative wound infection. (Tuazon, 1984).

Of patients with AD, 80- 100% are colonized with *Staph. aureus* (Hauser, *etal*., 1985, and Leung, *etal*., 1993). In contrast , *Staph. aureus* can be found on the skin of only 5-30% of normal individuals, mainly in interiginous areas. Colonization density is signification lower in healthy individuals than in patient with AD, and bacterial counts on unaffected skin are lower than on affected skin (Monti, *etal*., 1996). *Staph. aureus* often constitutes up to 80% of the normal flora in AD and large numbers of this microorganism seem to eliminate the lipophilic coryneform bacteria from the skin (Williams, *etal*., 1990).

The density of *Staph. aureus* on AD lesions has been shown to correlate with cutaneous inflammation (Williams, *etal*., 1990; Bunikowski, etal,2000 and Orwin , *etal*.,2001)

1-2-10-5- Role of *Staphylococcus aureus* in Atopic Dermatitis

1-2-10-5- 1- Prevalence of *Staph. aureus* in atopic dermatitis

Staph. aureus is found on the skin over 90% of patient with atopic dermatitis (Neuber, *etal*., 1993).Using electron microscopy, Morishita , *etal*., 1999) found *Staph. aureus* distributed on the surface of the epidermis as well as growing between layers of keratinocytes. Interestingly, staphylococcal toxins could be identified by immunoflorescence as deeply into the skin as on the inflammatory cells infiltrating into the dermis. In contrast, only 5% of normal subjects harbour this bacterium on their skin, and its localization is mainly in the nose and intertriginous areas. (Strange, *etal*., 1996)

The density of *Staph. aureus* on acutely inflamed AD lesion is frequently more than 1000 times higher than on nonlesional atopic skin. Clinical superinfection with *Staph. aureus* can reach 10^7 organisms per cm^2 on acute lesional skin. Honey-colored crusting, extensive serous weeping, folliculitis, and pyoderma indicate bacterial infection usually secondary to *Staph. aureus* in patients with AD.(Lever, *etal*., 1988; Skov, *etal*., 2000 and Breuer, *etal*., 2001).

1-2-10-5-2-Mechanism(s) for enhanced *Staph. aureus* colonization in Atopic dermatitis

The mechanism (s) leading to increased *Staph. aureus* colonization in AD are poorly understood. It is likely to result from a combination of processes, including disruption of skin barrier function from scratching, loss of certain innate antibacterial activities from changes in β -defensin level or reduced immune responses necessary for eradication and defense against bacteria, as well as changes in skin surface pH values toward alkalinity. (Mempel,*etal*., 1998 and Orwin ,*etal*.,2001). There has also been much interest in the potential role of lipid deficiencies in atopic skin since lipids have antimicrobial effects and an altered lipid content in the skin may lead to increased transepidermal water loss contributing to the dryness and cracked, brittle skin that predisposes to *Staph.aureus* colonization, varying according to the patient's genetic predisposition and environmental exposure.(Miller, *etal*., 1988; Imokawa, *etal*., 1991, and Leung, 2002)

Studies have demonstrated increased adherence of *Staph. aureus* to Keratinocytes from atopic skin. (Cole &Silverberg, 1986). The reason for increased binding of *Staph. aureus* to AD skin is not completely understood but is likely to be driven by atopic inflammation. This concept is supported by several observations:

First, it has been found that treatment with topical corticosteroids or tacrolimus significantly reduced the number of *Staph. aureus* found in atopic skin. (Stalder, *etal*., 1994, and Remitz, *etal*., 2001) Thus, it is very likely that atopic skin inflammation leads to the expression of attachment sites, which promote colonization of *Staph.aureus* (Leung, 2002).

Second, acute inflammatory skin lesions have more *Staph. aureus* than chronic skin lesions or unaffected atopic skin. (Hauser, *etal*., 1985). Scratching likely enhances *Staph.aureus* binding by disturbing the skin barrier, releasing proinflammatory cytokines, which upregulate extracellular matrix molecules known to act as adhesions for *Staph. aureus*. Alternatively breaks in the epidermal layer from scratching or skin dryness can expose underlying extracellular matrix molecules, which can serve as an anchor for adherence of *Staph. aureus* to the skin. (Foster & Hook, 1998 and Orwin ,*etal.*,2001).

Third, Cho, *etal*.(2001) found that Bacterial binding to frozen skin sections was significantly greater at skin sites with Th2-mwdiated inflammation than skin sites with Th1-mediated inflammation. Importantly, this increased bacterial binding did not occur in IL-4gene Knockout mice. Conversely when normal mouse skin was incubated *InVitro* with IL-4, as compared to interferon-γ , increased *Staph. aureus* binding occurred in skin explants treated with IL-4. These data indicate that IL-4 plays a crucial role in the enhancement of *Staph. aureus* binding to skin.

The microbial components responsible for adherence are termed “adhesions”. During the past few tears, a number of important staphylococcal adhesins (aside from protein A) have been identified, which are responsible for the initial interaction between *Staph.aureus* and matrix proteins in different tissues. (Leung, 2002). These include fibronectin-binding proteins A and B; clumping factor A and B, which are fibrinogen-binding; and collagen adhesions. (Foster & Höök, 1998). Importantly, it is well established that the tissue ligands for some of these staphylococcal adhesions, e.g., fibronectin, are modulated by proinflammatory cytokines, of interest, IL-4,but not interferon–γ, is known to induce fibronectin production by skin fibroblasts. (Postlethwaite , *etal*., 1992 and Noble , 1998).

The susceptibility of the atopic skin to colonization with *Staph. aureus* may have several causes . *Staph. aureus* cell wall contain receptors, the so-called “adhesions”, for epidermal and dermal laminin and fibronectin. Since the skin of patients with AD lacks on intact stratum corneum, dermal fibronectin receptor might be uncovered and increase the adherence of *Staph. aureus* (Cole & Silverberg, 1986, and Finegold, 1986). Fibrillar and amorphous structures have been traced between *Staph. aureus* cell and corneocytes, and may result in a bacterial biofilm that contributes to the adherence of *Staph. aureus* (Morishita, *etal*., 1999).Skin surface lipids, such as free fatty acids and polar lipids, have been shown to exhibit antibacterial activity. (Miller, *etal*., 1988). The observation that *Staph. aureus* penetrates into the intracellular spaces of the epidermis suggests that skin-surface lipids are deteriorated in patients with AD (Morishita, *etal*., 1999). Strikingly significant increases in the carriage of *Staph. aureus* were found in the anterior nares and hands of caregivers of children with AD compared to caregivers of healthy children, a fining which suggests transmission . (Williams, *etal*., 1998).

1-2-10-5-3- Role of Staphylococcal Virulence factors in Atopic Dermatitis

1-2-10-5-3-1- Superantigens

Recent studies suggest that one strategy by which Staph. aureus exacerbates skin inflammation in AD is by secreting a group of toxins that are known to act as superantigens

(Fig-5-) (Leung, 2000). These potent toxins bind directly without antigen-presenting cells (APC) such as macrophages or dendritic cell and to cytokine induced HLA-DR molecules on non professional APC such as keratinocytes (Kotzin, *etal*., 19993). The stimulation of T cells by superantigens results in the activation and expansion of lymphocytes expressing specific T-cell receptor Vβ regions (Leung, 2002).Such T cells may include autoreactive T cells that migrate to the target tissue containing the autoantigen recognized by that T cell and mediate damage via cytotoxic mechanisms or the secretion of proinflammatory cytokines (Leung, 2002).While all T cells, they frequently cause the expansion of different portions of the T-cell repertoire. Identification of specific T-cell receptor Vβ expansions can be useful in supporting the concept that tissue inflammation is mediated by superantigens(Leung & Schlievert, 1997 and Orwin ,*etal*.,2001).

A variety of observations support a role for superantigens in the pathogenesis of AD as follow:

1. AD severity correlates with presence of IgE antibodies to superantigens. (Nomura, *etal*., 1999)
2. Superantigens augment allergen-induced skin inflammation by activating infiltrated mononuclear cells and inducing mast cell degranulation.(Hofer, *etal*., 1999)
3. Superantigens induce dermatitis on application to skin by patch testing. (Strange, *etal*., 1996)
4. Patients recovering from toxic shock syndrome develop chronic eczema.(Bunikowski, *etal.,* 2000)
5. Superantigens induce the skin-homing receptor on Tcells (Akdis, *etal*., 1999)
6. Peripheral blood mononuclear cells from AD, as compared to normal controls, have higher proliferative responses to superantigens. (Michie & Davis, 1996).

Many recent studies evidenced role of staphylococcal superantigens in atopic dermatitis. First, over half of AD patients have *Staph. aureus* cultured from their skin that secrete superantigens such as enterotoxins A,B and toxic shock syndrome toxin-1(TSST-1) (Leung, *etal*., 1993; Bunikowski, *etal*., 1999, and Nomura, *etal*., 1999).

Other data suggested that superantigens can contribute to AD pathogenesis by increasing the frequency of memory T cells able to migrate to and be activated with in AD lesions. (Strickland, *etal*., 1999, and Bunikowski, *etal*., 2000)

Second most AD patients make specific IgE antibodies directed against the staphylococcal superantigens found on their skin (Nomura, etal; 1999).Basophiles from patients with IgE specified to superantigens release histamine on exposure to the relevant superantigen, but not in response to superantigens to which they make no specific IgE.(Leung, *etal*., 1993).These finding raise the intriguing possibility that superantigens induce specific IgE in AD patients and chronic mast cell degranulation *In Vivo* when the superantigens penetrate their disrupted epidermal barrier. This promotes the itch-scratch cycle contributing to the evolution of skin rashes in AD.(Nomura, *etal*., 1999, and Leung, 2002).

Third, a correlation has been found between the presence of IgE to superantigens and severity of AD.(Bunikowski, *etal*., 1999). Furthermore, colonization with superantigen – producing *Staph.aureus* is at greatest density in patients with IgE to staphylococcal superantigens (Nomura, *etal*., 1999). Skin-homing CLA+T cells have also been shown to respond to superantigen and contribute to esinophilia and IgE production in AD. (Akdis, *etal*., 1999).

Fourth, epicutaneous application of SEB to normal skin or unaffected AD skin has also been found to induce skin erythema and induration. (Strange, *etal*., 1996). In one

study, three of six AD subjects studied experienced a flare of their skin disease in the elbow flexure ipsilaterally to where the SEB was applied these observations provide direct evidence that superantigens can exacerbate and sustain skin inflammation with AD. (Skov, *etal.*, 2000)

A number of factors likely contribute to the induction of skin inflammation by superantigens. In this regard, superantigens can cause marked activation of the cells. These results suggest that superantigen-induced skin inflammation is T-cell dependent (Saloga, *etal.*, 1995).

1-2-10-5-3-2- Nonsuperantigenic toxins

Aside from superantigens, staphylococci can express other molecules that contribute to skin inflammation. Ezepchuk, *etal.*(1996) found that AD *Staph.aureus* isolation that failed to secrete superantigenic toxins produced α-toxin. All of these staphylococcal strains also expressed staphylococcal protein A. There were signification differences in the action of these molecules on keratinocytes. The superantigens TSST-1,SEA,SEB, and exfoliative toxin as well as protein A did not induce significant cytotoxic damage on keratinocytes. In contrast, α -toxin induced profound keratinocyte cytotoxicity that was time and dose dependent. The morphological and functional characteristics of cell death induced by α -toxin were consistent with cell necrosis, but not apoptosis. Lipoteichoic acid found in all *Staph.aureus* strains are also potent inducers of proinflammatory cytokine production by mononuclear cells. (Morath, *etal.,* 2001). Thus, a wide variety of staphylococcal products can have proinflammatory effects on the skin. (Bratton, *etal.,* 1995; Leung, *etal.,* 1995; and Vries, *etal.,* 1998).

Specific IgE to *Staph.aureus* cell wall components has been detected in up to 25% of patients with AD.(Neuber & König, 1992). Some authors found sensitization to staphylococcal cell walls to be associated with the severity of the disease. (Gabrielsen & Brandtzaeg, 1985). Bacterial cell wall components may also influence B cells directly, since costimulation of PBMC from patient with AD with IL-4 and teichoic acid or peptidoglycan led to pronounced increase of IgE synthesis and CD23 expression invitro. (Neuber & König, 1992).

1-2-11- Immunology of atopic dermatitis figures (6,7 and 8)

1-2-11-1- T-Lymphocytes

Activated T lymphocytes secrete a variety of cytokines, which have effects on the inflammatory reaction in lesional skin. (Werfel and Kapp, 2002). An important cytokine (Probably a major target of anti-inflammatory drugs like corticosteroids or macrolactams) is interleukin (IL)-2.IL-2 is a very efficient activator of surrounding resting T- lymphocytes, which may perpetuate the local cellular reaction. It may increase the clinical reaction since intradermal injection of this cytokine can cause intense pruritus. (Wahlgren, *etal.*, 1995 and Renz , *etal.*,2004)

Patients suffering from AD have increased levels of activated circulating T cells and increased levels of L-selectin and the secretory IL-2R, which are markers for lymphocytes activation and which correlate with disease severity. (Dworzak, *etal.*, 1999; and Shimada, *etal.*, 1999). In addition, Wu, etal (2000) described a significant reduction of the telomere length in all T cells subjects from atopic dermatitis patients compared with normal individuals. The authors concluded that the increased telomerase activity and shortened

telomere length indicates that T lymphocytes in atopic dermatitis are chronically stimulated and an increased cell turnover *In Vivo*.

The number of CD4+ cells is increased, and CD8+ suppressor/ cytotoxic lymphocytes are decreased in peripheral blood. However, psychological stress has recently been shown to lead to significantly higher increases in the number of circulating CD8+ T lymphocytes in AD patients compared to healthy controls. (Schmid, *etal*., 2001 and Renz , *etal*.,2004).

1-2-11-2-Mechanisms of T-cell adhesion and activation in Atopic Dermatitis

T- Lymphocytes circulate through three different types of compartments in the human body that can be divided into primary, secondary, and tertiary lymphoid organs. In order to arrive in these organs the lymphocytes express adhesin molecules which are more or less specific for the target organ. The mechanism of lymphocyte invasion into the tissues is thought to resemble that of monocytes and neutrophils, being a multistep process involving attachment and rolling through selectin-carbohydrate interaction, activation through chemoattractant – receptor interactions, and firm adhesin through intergrin-immunoglobulin family interaction. (Springer, 1994).

More than 80% of skin-infiltrating T- Lymphocytes express the cutaneous lymphocyte-associated antigen (CLA) molecules. This molecules has been intensively studied with respect to inflammatory skin responses in men . (Akdis , *etal*., 1997)

System activation of T cells in AD is supported by the observation that these patients possess increased numbers of activated CLA-bearing T cells in the circulation. (Santamaria, *etal*., 1995 and Akdis , *etal*., 1997)

The dermal cellular infiltrate in AD mainly consists of $CD4^+$ and $CD8^+$ T cells with a CD4/CD8 ratio similar to peripheral blood levels. (Akdis, *etal*., 1999). In other studies, $CD8^+$ CLA^+ T cells were demonstrated to be as potent as $CD4^+$ CLA^+ T cells in induction of immunoglobulin E(IgE) and prolonged eosinophil survival . (Akdis, *etal*., 1999). Several factors leading to T cells activation in AD, including aeroallergens, food allergens, and superantigens, have been emphasized. Normally, allergen-specific T cells responses in food and aeroallergen allergy are confined to $CD4^+$ T cells. (Akdis, *etal*., 2002).

1-2-11-6- Immune responses in AD skin

Clinically unaffected skin in AD is not normal. It frequently manifests increased dryness and a greater irritant skin response than healthy control (Leung, *etal*., 2004). Unaffected AD skin contains a sparse perivascular T cell infilterate not seen in normal healthy skin. Immunological pathways in AD illustrated in **Figure(7)**. The cells circulating in the peripheral blood of AD patients result in elevated serum IgE and eosinophils. These T cells express the skin homing receptor, CLA, and recirculate through unaffected AD skin where they can engage allergen – trigged IgE^+ LC_s and mast cells (MC_s) that contribute to Th2 cell development. Skin injury by environmental allergens, scratching, or microbial toxins activates Keratinocytes to release proinflammatory cytokines and chemokines that induce the expression of adhesion molecules on vascular endothelium and facilitate the extravasation of inflammatory cells into the skin. Keratinocyte-derived thymic stromal lymphopoietin (TSLP) and DC-derived IL-10 also enhance Th2 cell differentiation. AD inflammation is association with increased Th2 cells in acute skin lesions, but chronic AD results in the infiltration of inflammatory $IDEC_s$, macrophages (MØ), and eosinophils. IL-12 production by these various cell types results in the switch to a Th1-type cytokine milieu associated with increased IFN-γ expression.(leung, *etal*.,2004)

There is an increased number of IgE-bearing LCs and IDECs in the epidermis, and macrophages dominate the dermal mononuclear cell infiltrate .(leung, *etal*., 2004). Eosinophils also contribute to the inflammatory response ,and T cells remain present, although in smaller numbers then seen in acute AD (Novak and Bieber, 2004) .Chronic AD lesions have significantly fewer IL-4 and IL-13 mRNA-expressing cells, than in acute AD .(Novak, *etal*., 2003) Recent studies suggest that collagen deposition during chronic AD is due to increased gene expression of the profibrotic cytokine ,IL-11.(Toda, *etal*., 2003).

1-2-11-7- **The immunopathology of atopic dermatitis**

Genetic and environmental factors induce a complex series of cellular interactions leading to the symptoms and signs of AD.**(figure-8-)** (Spergel & Schneider, 1999). One potential scenario is that langerhans cells, which are surface IgE positive, present antigen to Tcells leading to their activation and release of cytokines, i.e. IL-1,IL-6 and TNF-α (Jeong, *etal*., 2003). Simultaneously, physical trauma (scratching) causes the keratinocytes to secrete similar cytokines , which in turn attract and activate $CD4^+$ T lymphocytes. Mast cells are also present and upon activation by cross-linking of IgE or other mechanisms, can release histamine and mediators upregulation adhesions molecules, which in turn may recruit additional inflammatory cells including Tcells and eosinophils (Kagi, *etal*., 1994, and Akdis & Akdis , 2003)

The Tcells appear to play a central role in the process. T-cells infiltrating the skin lesion express high levels of cutaneous lymphocyte antigen (CLA), which functions as a skin homing receptor for T lymphocytes by binding to E-selection. (Matsuda, *etal*., 1997). PBMC show increased IL-5 expression and TH2-like phonotype in $CD4^+$ and $CD8^+$ cells in patients with AD. However, the frequency of these allergen specific Tcells that proliferate to *Dermatophagoides pteronyssinus* are between 0.02 and 0.7% suggesting AD lesion also contain no- specific inflammatory cells.(Spergel & Schneider,1999).

Other studies, however, have revealed that in the chronic eczematous AD skin lesions, the expression of the TH-1 cytokine, IFN-γ predominates compared to TH2 phenotype in the acute lesions. This has also been confirmed by patch testing , when the majority of Tcells were found to express IFN- γ m RNA and to secrete IFN-γ protein, either alone or in combination with IL-4 in last lesions . these results suggest that the chronic lesions are not completely TH2, but more likely a combination of TH2 and TH1 phenotypes. (Ruzicka & Ring,1987;Okano,*etal.,*1996, and Lintu, *etal.,*1997).

The activated TH2 cells are seen in the acute lesions and probably in the chronic lesions and secrete IL-3, IL-4, IL-5, IL-6, IL-10, IL-13 and GM-CSF . these cytokines promote & cell responses, upregulation of IgE receptors on langerhans cells and down regulation of TH1 activity and IL-1 receptors of monocytes. (Leung, 1992). The latter effects may account for the lack of delayed-skin reactivity and the cutaneous anergy seen in most patients with AD. Two cytokines have critical roles, IL-4 for invitro IgE synthesis, and IL-5 promotes the invitro differentiation and survival of eosinophils. IL-4 can activate keratinocytes, which in turn have enhanced ability to estimate Tcells (Leiferman, 1994).

The role of IFN- γ in AD is more controversial IFN- γ is decreased in many studies but is seen in biopsy of chronic lesion. It is possible to postulate that IFN-g is increased locally and decreased peripherally. (Spergel & Schneider, 1999) .IFN- γ may have a role in the chronic lesions by activating neutrophils, macrophages, endothelial and epithelial cells , fibroblasts in addition to inducing expression of HLA-DR on keratinocytes. (Leung, *etal.,*2004). Keratinocytes production of GM-CSF may also contribute to the establishment and chronicity of AD lesions, in particular to increased number, sustained activation, and enhanced antigen-presenting functions of dendritic cells. Therefore , the pattern of

cytokines expressed locally is critical in modulating the nature, extent and persistence of the inflammation in AD. Another mechanism for the persistent inflammation is enhanced survival of inflammatory cells.(Hamid, *etal.,*1994 and Leung,1995).

1-2-11-8- Immunological differences between intrinsic and extrinsic types of atopic dermatitis

Two forms of AD have been delineated : Extrinsic AD (EAD) (allergic) form associated with IgE- mediated sensitization involving 70-80% of the patients, and an Intrinsic (IAD) form, these subgroup of AD patients with normal IgE levels and without specific IgE- medicated sensitization involving 20-30% of the patients , also termed non-atopic dermatitis, non-allergic form of AD, and atopiform dermatitis. (Akdis &Akdis, 2003).

Immune effector mechanisms in extrinsic- type dermatitis (EAD) and intrinsic-type (IAD) show in **Figure(6)**. In the peripheral blood of EAD patients, both $CD4^+$ and CD8+ subsets of CLA^+ $CD45RO^+$ Tcells are in an activated state ($CD25^+$,CD40 –Ligand (L^+) , $HLADR^+$). (Kagi, *etal.,*,1994, and Akdis & Akdis,2003) . Mainly the TH1-like compartment of them undergoes Fas-mediated activation-induced cell death(AICD). In contrast, Tcells infiltrating the skin of AD patients-despite expressing both Fas and FasL do not show any apoptosis, because they are protected from apoptosis by cytokines and ECM proteins. These T cells secrete IFN- γ, which up- regulates Fas on keratinocytes and renders them susceptible to apoptosis in the skin.(Schmid, *etal.,* 2001, and Bos,2002). Keratinocyte apoptosis leading to spongiosis is induced by FasL expressed by activated Tcells. Keratinocytes undergoing apoptosis in acute eczematous lesions release IFN- γ induced chemokines (IP-10, Mig and ITAC) that attract more $CXCR3^+$ T cells towards the epidermis, which may further augment the inflammation and keratinocyte apoptosis. (Akdis, *etal.,*1999). In EAD, T cells isolated from skin or CLA^+ $CD45RO^+$ T cells from peripheral blood secrete high levels of IL-5 and IL-13, and are therefore capable of prolonging eosinophil life span and activating B cells for CD23 expression and inducing IgE production in EAD. (Jeong,*etal.,*2003). In IAD no specific IgE , but IgG is found in the circulation against allergens. Epidermal dendritic cells (EDC) and langerhans cells (LC) express high levels of $FC_\varepsilon RI$ in EAD. (Oppel,*etal.,*2000,and Bos,2002).

1-2-12- The epidemiology of Atopic Dermatitis

The prevalence of AD varies markedly across the world. Williams, (1999) have shown that prevalence varies from just above 1% to just below 20% in children aged 6-7 years.

AD is increasing in frequency overtime. A study of Scottish school children showed a prevalence of 5.3% in 1964 and 12% in 1986 reasons suggested for the increasing prevalence include : Decreased rates of breast – feeding, Earlier introduction of weaning foods, wide spread use of food additives, changes in the formulation of infant formulas, and environmental factors favouring the expression .(Williams, 1999)

The effects of AD range from dry skin and mild irritation to a generalized, severe, disabling skin condition. It is therefore necessary to some how express severity as part of an epidemiological description of the condition.(Daniels & Harper, 2002).

AD is a genetic disorder based on the interactions between an unknown number of genes and environmental factor. In a family with an affected child there is usually a family history for eczema or one of the other atopic disorders, asthma and rhinitis. Candidate genes include those that have been shown to influence immunoglobulin E (IgE)

responsiveness : receptors for IgE (chromosome 11); the interleukin 4/5 cluster (chromosome 5); interleukin 4 receptor (chromosome 16); HLA –DR (chromosome 6) and the T-cell receptors α / δ (chromosome 14) , and those that are associated specifically with AD : mast cell chymase (14q11), SPINK5, the Netherton's gene (5q 31) (Cookson & Moffatt , 2002).

AD affects up to 20% of childhood population in 65% AD had resolved by years of age, and in 74% AD had resolved by 16 years of age. (Cooper, 1994 and Charman & William , 2002).

In recent study generated complete data for 256,410 children aged 6-7 years in 90 centers and 458,623 children aged 13-14 years in 153 centers. The 12-month period prevalence estimates for the 6 to 7 year age group ranged from under 2% in Iran to over 16% in Japan and Sweden. In the 13-to 14- year of age group, disease prevalence ranged from less than 1% in Albania to over 17% in Nigeria. Many of these variations of results of ISAAC(Internation Study of Asthma and Allergies in Childhood) cannot be completely explained known risk factors or established hypotheses concerning the disease (Charman & Williams, 2002) .

FIGURES (1) : CLINICAL FEATURES OF ATOPIC DERMATITIS (ECZEMA) IN VARIOUS LOCATIONS OF DIFFERENT AGES .

NOTE : ALL PICTURES WERE PUBLISHED UNDER DIRECT PERMISSIONS FROM THEIR PUBLISHER / OR AUTHORS .

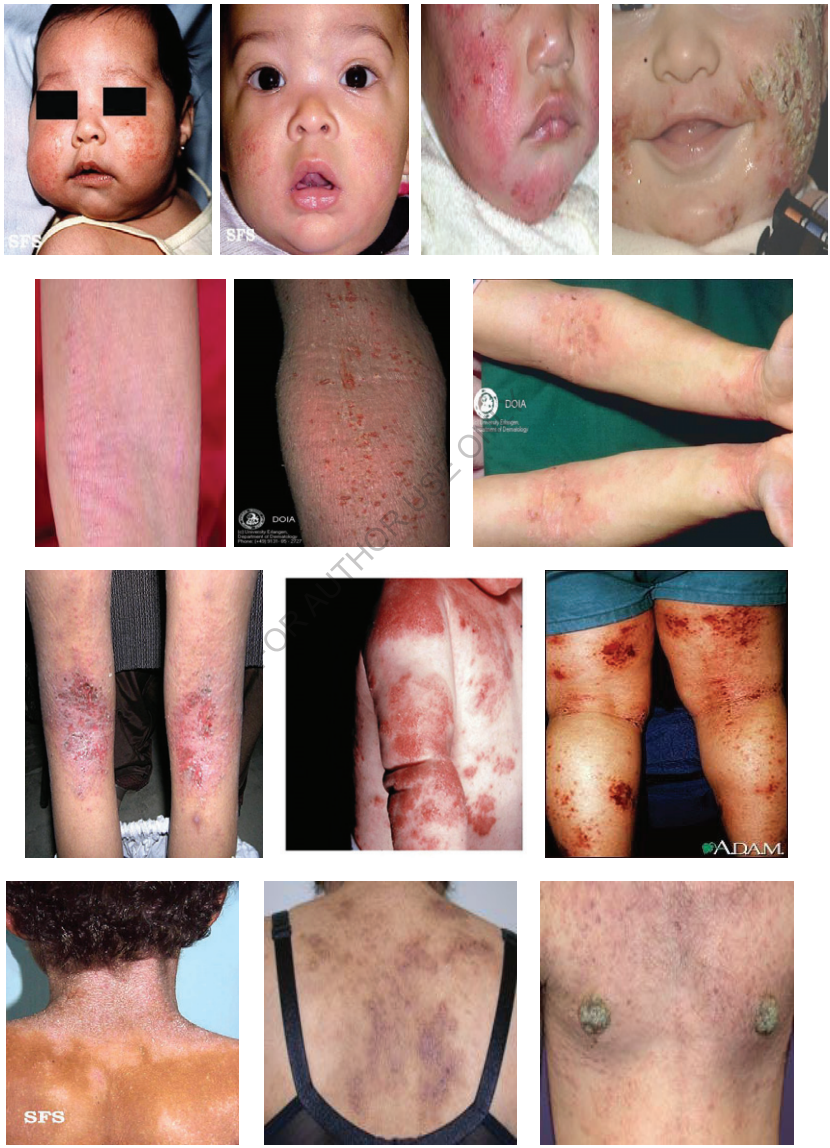


FIGURE (2) : THE STRUCTURE OF HUMAN SKIN . (HEYNEN , 2004)

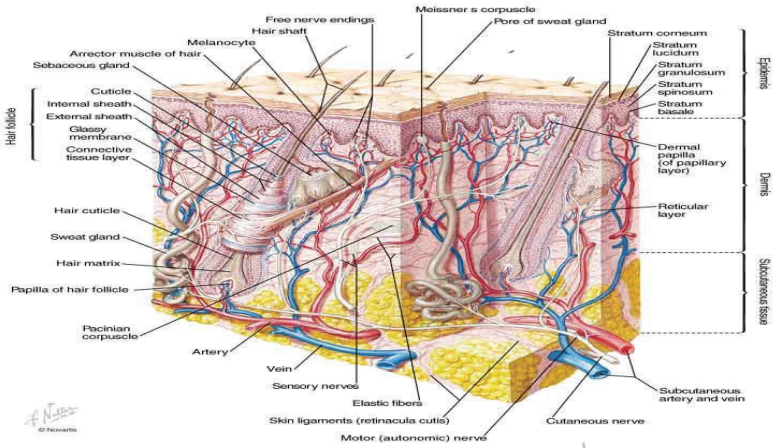
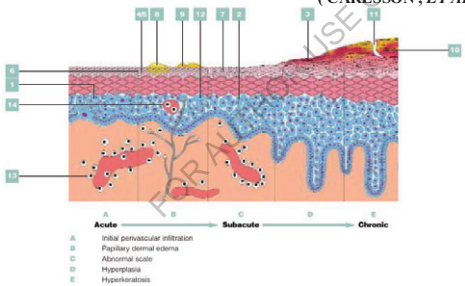


FIGURE (3) : SKIN PATHOLOGICAL FEATURES OF ATOPIC DERMATITIS (HEYNEN , 2004) .

FIGURE (4) : DIFFERENCES IN BINDING OF ANTIGEN AND SUPERANTIGEN . (CARLSSON , *ET AL* ., 1988)



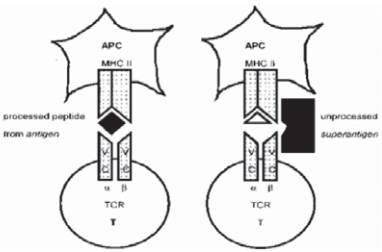
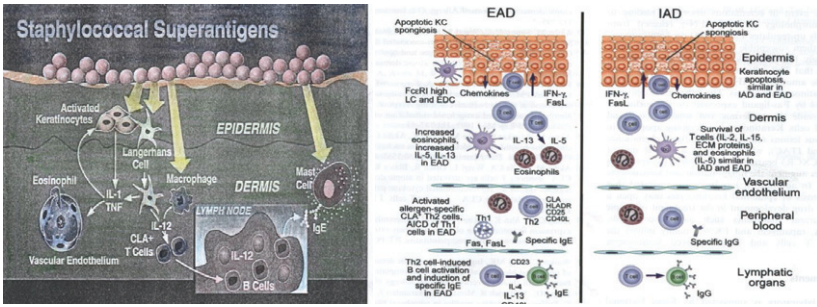


FIGURE (5) : ROLE OF STAPHYLOCOCCAL ERANTIGENS IN ATOPIC DERMATITIS . (LEUNG , 2000)

FIGURE (6) : IMMUNE EFFECTOR MECHANISMS IN EXTRINSIC – TYPE DERMATITIS (EAD)AND INTRINSIC – TYPE (IAD).(AKDIS & AKDIS , 2003)



FIGURES (7) : IMMUNOLOGICAL PATHWAYS IN ATOPIC DERMATITIS . (LEUNG , *ET AL* ., 2004)

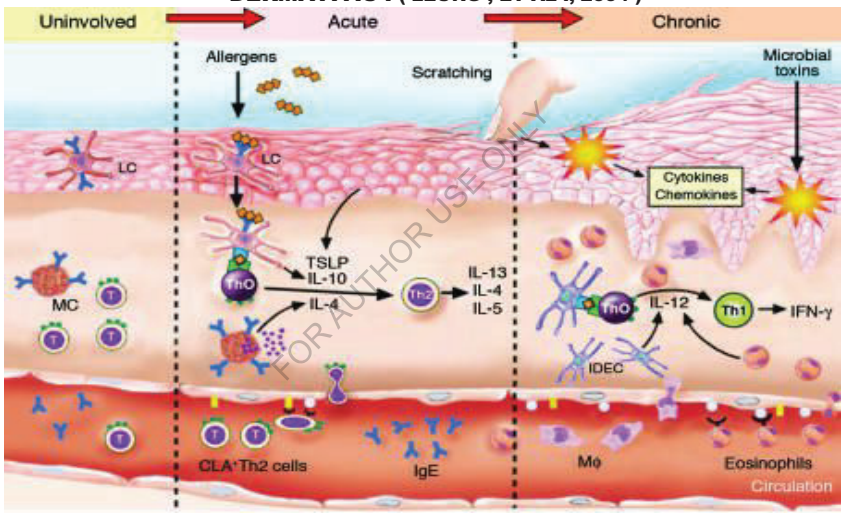
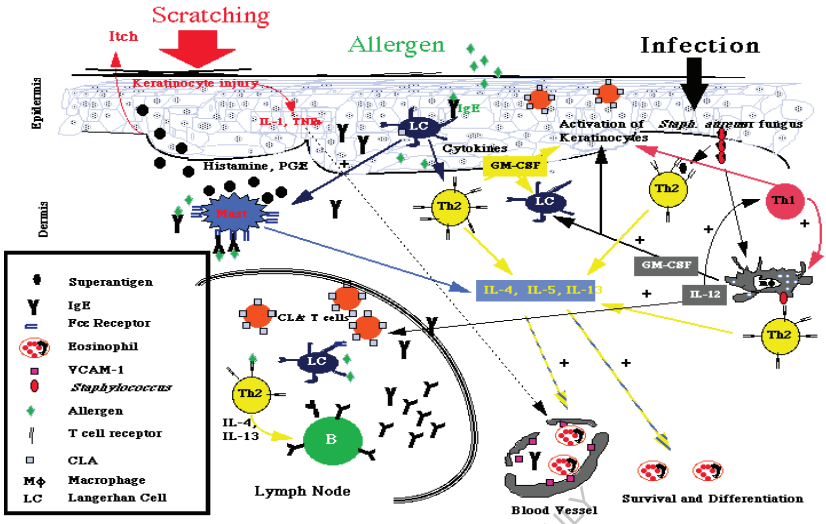


FIGURE (8) : IMMUNOPATHOLOGY AND CELLULAR INTERACTIONS OF ATOPIC DERMATITIS . (SPERGEL & SCHNIDER , 1999)



Chapter two:
MATERIALS & METHODS

2-1- samples collection

2-1-1- patients

A total of (484) patients (211 males and 273 females) of various age groups were included in this outpatients based study. The patients were suffering from atopic dermatitis, attending the dermatology outpatient clinics of the main hospitals in Basrah providence (outpatient based study) : Alsadr teaching hospital , Basrah General hospital , port General hospital . AL- Shefaa General hospital , in addition to a specialized private clinics . The patients were examined , and diagnosed as atopic dermatitis cases under supervision of dermatologists based on criteria of (Hanifin & RajKa , 1980; Spergel & Schneider , 1999 and Stanway , 2005). The study was carried out during a period from November 2003 to July 2005.

2-1-2- The grouping of patients

The patients male & female were grouped into five groups
According to (Falk , 1993; Herd , *etal.,* 1996; Nishioka , 1996 and Charman & Williams , 2002).
These groups are:

1 Infantile group: less than two years .
2 Childhood group: from 2 to < 11 years .
3 Adult hoot group : more than 11 years , and then

subdivided in to : group (3) : 11 to < 20 years .
group (4) : 20 to < 30 years .
group (5) : over than 30 years .

Control group

A total of (100) healthy individuals were randomly collected (without any AD features , skin infections and immunological disorders) to compare with AD patients in hematological and immunological studies.

2-1-3- Sampling and specimens

1-skin swabs were taken from AD patients(Eczematous lesions and nearest healthy area (control), they were saturated by brain heart infusion broth (BHIB) (Oxoid) , and transported to the laboratory for immediate processing .(Forbes, etal., 1998).
2- Blood samples : 10 ml of blood was collected by venous puncture in a suitable tubes according to (Fischbach , 2000) from patients and control groups. Blood samples were taken for hematological and immunological investigations .
3- Skin biopsies from eczematous and nearest healthy area of patient's skin were taken for histopathological study according to (Fischbach , 2000 and WHO , 2002).

2-2- Bacteriological study

2-2-1- Primary isolation

Skin swabs from both eczematous lesions and healthy area were cultured on primary isolation media: blood agar base(Oxoid) **,** MacConkey agar **,** and nutrient agar (Himedia) (Forbes ,*etal*., 1998). And then incubated aerobically at 37ºC for 24 – 48 hours .Samples that cultured on chocolate agar in addition to nutrient agar were incubated anaerobically in candle jar at the same temperature and period mentioned above

2-2-2-Bacterial count

Enumeration of a total bacterial counts per cm ^{2} of same above sites were carried by using a serial dilution technique of nutrient broth(Himedia) and then culturing on nutrient agar. (Forbes , *etal*., 1998).

2-2-3- Identification techniques

Four types of **Api technique** (BioMerieux **,** France)(**picture-1-)** were used for rapid identification of various bacterial isolates based on enclosed instructions of supplied company :

Api staph : Identification system for staphylococci
Api 20 strept : Identification system for streptococci
Api 20 E : Identification system for Enterobacteriaceae and other Gram – negative rods
and Api 20A : Identification system for anaerobes:

Mannitol salt agar and Staph 110 (Himedia) were used as a selective media and some specilized biochemical tests were done for confirmation the diagnosis of *Staphylococcus aureus* (Forbes , *etal*., 1998 ; Garrity , etal., 2001 and Brooks ,*etal*., 2004) .

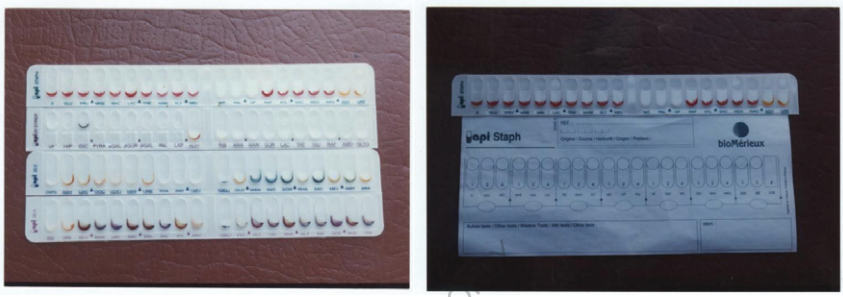
Bacteriological study was done for (286) AD patients.

2-2-4- Antibiotics Susceptibility

Thirteen antibiotics (Himedia , India) commonly prescribed for treatment of atopic dermatitis were used to test antibiotics susceptibility of *Staph.aureus*:

Amoxycillin / Clavulanic acid(20 / l0 mg) (AC)
Bacitracin (10 U) (B)
Cephalothin (30 mg) (Ch)
Chloramphenicol (30 mg) (C)
Clindamycin (2 mg) (Cd)
Co – trimoxazole (trimethoprim / sulphamethaxazole)
(1.25 / 23.75 mg) (Co)
Doxycyclin hydrochloride (30mg) (Do)
Erythromycin (15 mg) (E)
Gentamicin (l0 mg) (G)
Methicillin (5 mg) (M)
Rifampicin (5 mg) (R)
Tetracycline (30mg) (T)
and Vancomycin (30 mg) (Va)

PICTURE (1) : **API SYSTEM (BIO MERIEUX , GERMANY) USED FOR IDENTIFICATION OF BACTERIAL TYPES ISOLATED FROM NORMAL AREA ANDECZEMATOUS LESIONS OF AD PATIENTS .**
LEFT : ALL API SYSTEMS USED IN THE STUDY INCLUDES : API STAPH , API 20 STREPT , API 20 E , API 20 A .
RIGTH : API STAPH WITH CHART OF RESULTS .



PICTURE (2) :
LEFT : PLATES OF VARIOUS TYPES OF IMMUNOGLOBULINS (IgA , IgG , IgM) AND COMPLEMENTS (C3 , C4).
RIGTH : ANTIBODY – ANTIGENE REACTIONS (PRECIPITATION)

2-3- Hematological study

Primary hematological examination (Hb% , ESR mm/hr , total RBC , WBC , Platelets and differential leucocytes (WBC) counts were carried out manually according to a standard basic methods (Fischbach , 2000), then Repeated and confirmed by using autoanalysis instrument (Beckman Coulter , T- 660 , USA) to measure all above mentioned and other hematological parameters .

Hematological examination was done for (374) AD patients.

2-4- Immunological study

2-4-1-Measurment of Immunoglobulins & components of complement concentrations by single radial Immunodifusion test (SRID)

A kits of radial immunodifusion plate (Biomagherb , Tunisia) **(picture-2-)** were used to determine the concentration of immunoglobulins ; IgA IgG , and IgM , and C3 & C4 components of complement for **(212) AD patients.**

Each plate (12 test plate) contains mono specific antiserum directed against IgA, IgG , IgM , C3 and C4 which was incorporated in an agarose gel layer. 5 ml of serum from AD patients and / or control samples placed in wells then incubated at room temperature for 48 hours to IgA , IgG , C3 and C4 plates, while IgM plates were incubated for 72 hours. End point of diffusion is indicated by a sharp precipitation ring , which is achieved when incubation time is finished. Readings were done at this time. The diameters of each ring were measured directly by using magnifying lens with micrometers scale. The diameter of the ring was related to antigen concentration and the results were evaluated by using reference standard table that is packaged with the kit instruction method supplied by (Biomagherb – Tunisia).

2-4-2- Measurement of the concentration of total IgE

Quantitative determination of total concentration of IgE was done for **(178)AD patients** by total IgE microplate Elisa kit (Biomagherb) , which is a two – site enzyme linked immunosorbent assay . It could be summarized assay procedure as fallow:

1- 20 ml of standards labeled in duplicate , control and 20ml of patient sera or control sera were added to 100ml of assay buffer Elisa plate wells , and 20ml of 0 standard to blank substrate was added to blank well .
2- Incubated in an incubator for 90 minute 37^{o}C after homogenized at 300rpm (horizontal shaking)
3- Three times washing by 300ml of wash solution in to each well by auto strip washer (ELX50 Bio- tek. Inst.)
4- 100ml of conjugate(anti-IgE alkaline phosphatase conjugate) was added in to each well and incubated for 90mins at 37 ^{o}C then washed same as steep 3
5- 100ml of chromogen (PNPP as a substrate) was added into each well and incubate for 30 mins at room temperature in the dark.(PNPP: Para Nitro- Phenyl-Phosphate)
6- 100ml of stop solution (NaOH, 2N) was added into each well.
7- Universal microplate ELISA Reader (Bio-tek. Instruments, Inc. mod.ELX800)was used to read the result against blank well of all strips at 405nm, and.

8- The total IgE concentration was evaluated in(IU / ml) by semi log paper.
Allergy may be detected according to total IgE concentration as follow :

Lower than 20 IU/ ml	Allergy not propable
Between 20 – 100IU / ml	Allergy questionable
Higher than 100 IU / ml	Allergy very probable

The Assay was done according to instruction of suppling company (Biomagherb - Tunisia).

2-4-3- Measurement of Allergen – specific IgE concentrations

Ultra EAST or Enzyme Immunoassay (EIA) method was used for the semi quantitative determination of allergen – specific IgE concentration in sera of **(178) AD patient** and control.

Assay procedure can be summarized as a following steps according to Instructions supplied by Biomagherb -Tunisia.

1 Allergen labeled discs (6mm in diameter) were placed into wells except the substrate blank well .The discs are three types:

a- Reference disc (D1)
b- Commercial disc supplied by Biomagherb
c- Manually made up disc by cork piercing (no 2) to make 6mm disc saturated by bacterial antigens as allergens (illustrating later).

2 50 ml of reference calibrator A- H was added directly onto the disc (D1) and 50ml of AD patients and control sere were added directly onto allergen disc , then shacked for 30secs at 200 rpm (microplate horizontal shacker) and incubated for 90 mins at room temperature (20-25^{o}C)

3 Six times washing by 0.3 ml of diluted washing solution by autostrip washer ELX50 , Bio – tek. Inst.)

4 50 ml of the anti – IgE conjugate was added into the disc in each well except substrate blank well , then shacked for 30 secs at 200 rpm and leaved overnight at room temperature .

5 Washings were Repeated as in step -3-

6 50 ml of the working substrate solution (PNPP solution) was added onto all wells including substrate blank well , then incubated for 20 mins in the dark at room temperature.

7 200 ml of stop solution (2N NaOH) was added onto all wells including blanks.

8 200 ml of supernatant transferred to another microtiter plate and the final results measured directly by reading of universal microplate Elisa reader (Bio – tek. Instrument , Inc. Mod. ELX800) At 405 nm with reference at 600-650 nm.

Calculation :

I- The absorbances average were calculated for D, C, B, A and H reference wells .
II- The results of the positive & negative control , AD patient and control samples on all discs were calculated by following method:
Compared the absorbances of each control and AD patients samples with the absorbances for the reference sera D, C, B, A, H. Assign the class value to the sample as flow :

Class	Count rate	IgE titer (EU/ml)	specific IgE concentration
5(+++++)	> H	>52.5	super high
4(++++)	A-H	17.5 – 52.5	very high
3(+++)	B-A	3.5 – 17.5	high
2(++)	C-B	0.7 – 3.5	moderate
1(+)	D-C	0.35 – 0.7	low

0	<D	<0.35	non detectable

Allergens that studied against specific- IgE were:
C1: pencilloyl G , **E1** : cat epithelium , **E3** : Horse epithelium , **E4** : Cow dender , **E7** : pigeon droppings , **E75**: chicken Feathers , **F1**: Egg white , **F2** : Milk , **F3**: codfish , **F9** : Rice , **F12** : pea , **F15** : white bean , **F24**: shrimp , **F25** : tomato , **F29** : banana , **F35**: potato , **F47** : Garlic , **F75** : yolk , **F81** : cheddar cheese , **F83** : chicken , **F108**:onion , **G1**: sweet vernal grass, **G7**: common reed , **G15** : wheat , **I6**: cockroach(German) , **M1***: Pencillium notatum*,**M2**: *Cladosporium herbarum* , **M3**: *Aspergillus fumigatus*, **M5**: *Candida albicans*, **M19**;*Aspergillus versicolor* ,**T9**:olive, **T11**: plane tree, **T204**: pepper tree(*Schinus molle*) , **U73**:silk, **U81**: cotton cultivated, **U82**: sheep's wool, **U83**: Formaldehyde, **W15**: scale, and **W28**: Rose.
In addition to above mentioned allergens, extracted bacterial antigens(as allergens) were tested against specific IgE (extraction illustrated later):
SA1: enzy: *Staph. aureus* 1 (Exotoxin as superantigen) ,
SA1: all: *Staph. aureus* 1(outer antigen as allergen) ,
SA1. Body: *Staph: aureus* 1(extracted body) ,
AS2. all: *Staph: aureus* 2(Allergen) ,
SA3. all: *Staph: aureus* 3 (Allergen) ,
Se.all: *Staph.epidermidis* (Allergen) ,
Stfe.all: *Sterpt.faecalis* (Allergen) ,
Stpyall: *Sterpt.pyogenes* (Allergen) ,
pr.ac.all: *Propionibacterium acnes* (Allergen) ,
pseu.all: *Pseudomonas* (Allergen) ,
and **E.coli.all**: *Escherichia coli* (Allergen) ,

2-4-4- Immunophenotyping analysis
(Measurement of cluster of differentiation (CD) antigens)

Since, there are no detailed previous studies interested in CD markers or antigens in our country , so , we give a full description about CD markers procedure according to the instructions supplied by (Bio source Int . Belgium) (BCSH ,1994 and WWW.Biosource.Com,2002)

2-4-4-1- Blood collection

Five mls of venous blood were collected by a septic venipuncture , from **(108) AD patients** and the control group , in a sterile glass tubes containing 10 IU / ml of sodium heparin or EDTA anticoagulated tubes.

2-4-4-2- Separation of mononuclear (MN) cells

For cell marker studies it is necessary to separate the MN cell fraction containing lymphocytes and to exclude red cells and granulocytes . Isolation of mononuclear cells was performed by density gradient centrifugation using LymphoPrep. This is an aqueous solution of the proper density, viscosity and osmotic pressure for use in a simple , rapid lymphocyte isolation procedure.

Principle of procedure

Anticoagulant . treated blood is layered on the lymphocyte separation medium(LymphoPrep) and centrifuged .Differential migration during centrifugation results

in the formation of layers containing different cell types . The bottom layer contains red cells which have been aggregated by the LymphoPrep and therefore , sediment completely. The layer immediately above the red cells contains mostly granulocytes. Because of their low density, the lymphocytes are found at the interface between the plasma and the LymphoPrep with other slowly sedimenting particles (platelets and monocytes). The lymphocytes and then recovered from the interface and subjected to a short washing step with balanced salt solution to remove any platelets , lymphoprep , and plasma.

procedure for Isolation of MN cells

1- 4mls of lymphocyte separation medium (LymphoPrep) were dispensed into a 10 mls siliconized glass centrifuge tube.
2- Carefully layer (drop by drop) 2-4 mls of diluted blood sample on a top of the separation medium.
3- After centrifugation at 400× g for 30 mins at 18-20ºC , mononuclear cells would form a grey colored at the interface of the blood plasma and the separation medium.
4- The upper layer drew off by using a clean Pasteur pipette, care should be taken not to disturb the MN cells.
5- A clean Pasteur pipette used to aspirated the layer of MN cells. Avoid aspirating any of the reminant separation medium as this can interfere with subsequent washing and cause contamination with granulocytes.
6- The MN cells was transferred to a clean centrifuge tube.
7- Hank's solution or PBS was Added at least 3 volumes to the lymphocytes in the centrifuge tube. Suspend the cells by gently drawing them in and out of a Pasteur pipette.
8- The MN cells suspension centrifuged at 100× g for 10-15 minutes at 18-20ºC. Aspirate the supernatant and discard . Repeat(7) and(8) three times.
9- The cells were resuspended in hank's solution. Adjust the volume of the MN cell concentration of 1-2× 10^7 cells / ml.

2-4-4-3- Cells count and viability

2-4-4-3-1-Cells count

10ml of well mixed cell suspension were pipetted into a 12mm× 75mm plastic reaction tube and mixed with 10ml trypan blue . A neubauer counting chamber was filled . And any excess fluid wiped off from under the coverslip and the number would counted in the large middle square. The cell count was calculated by :

Cell no x 2(dilution factor)x10000 per mls (center sq.)

2-4-4-3-2-Cells viability

Lymphocyte viability was determined by the trypan blue exclusion test. Trypan blue dye is taken up by dead cells. Thus cells that appear blue in colour are dead cells and not included as a part of the cell count .Percentage of viable cells are thus calculated by :

$$\% \text{ viability} = \frac{\text{Live cells}}{\text{live cells + dead (blue)cells}} \times 100$$

More than 95% of the lymphocyte viability was ensured to perform the immunophenotyping analysis (Abuhmood, 2003).

2-4-4-4- Immunoflorescence

principle

the cells are incubated with antibodies which are directly labeled with fluorochromes. After wards, washed cells are viewed with a fluorescence microscope under cover slip.

Procedure (BCSH,1994 and Biosource,2002)

A panel of five small plastic reaction tubes was prepared corresponding to the number of McAb reagents and negative controls used. In each of five tubes :

1- 100 µl of MN cell suspension containing (1-2x10^6 cells) was placed .

2- 50 µl of the McAb reagents and negative controls to the corresponding tubes was added.

* Fluorescent monoclonal antibodies (McAb) of known specificity and concentration were used . The following directly labeled McAb (goat-antimouse Ig) conjugating with(fluorescence –iso- thiocyanate) (FITC) (supplied by Biosource Int. Belgium) were used :

A- i. Anti –**CD_3** – McAb
ii. Anti –**CD_4** – McAb
iii. Anti –**CD_8** – McAb
iv. Anti –**CD_{19}** – McAb

B- negative control (isotype control antibodies : were setup by replacing the McAb with a mouse Ig of the same isotype, used as non specific staining controls in all cases .

3- The cell suspension was mixed well and incubated the mixtures for (30 mins – 1hour) at 4ºC in the dark.

4- After incubation, the cells washed twice in Hanks solution and centrifuged at 2000 rpm for 5 min in each .

5- All supernatants removed, and a pellet will leaved of labeled cells in 50 – 100 µl of washing medium.

6- 1-2 drop of PBS /glycerol (1 :1) (as amounting medium) was added .

7- The mounting on a clean glass slide and cover with coverslip, then sealing with nail varnish were carried .

8- The fluorescence microscope was used for examining.

9- When cells were left overnight one drop of 2% formalin was added to the labeled cell suspension.

Evaluation

Immunoflorescence was determined by fluorescence microscope (Leitz). A reaction was considered positive when the cells have multiple fluorescent dots on the membrane or homogenous bright green membrane fluorescence. At least 200 cells were counted in each case to assess the immunoflorescence. Less than 5% of the cells were positive for the negative control. A marker was considered positive when expressed in over 30% of cells above the negative control. (BCSH,1994) .

2-5-Isolation,Purification,Identification and characterization of Staph. aureus (Sa1) Exotoxin (as a superantigen)

Because , the highest majority of Staph. aureus prevalence in the most of AD cases, then , all followed experiments were carried on these bacterial type :

2-5-1- Primary screening :

Five isolates of *Staph aureus* were selected according to highly prevalence of disease course and severity of Atopic dermatitis associated with these bacterial type, and the antibacterial activity against four standard strains of bacteria was studied by culturing *Staph.aureus* with each of these bacteria separately (Melconian , *etal.*,1983 and

Schmauder,1997).

***Staph.aureus* strain no.1 (Sa1)** was selected according to the results of above mentioned step, then cultured on fermentation media (Yeast-Malt Extract Agar (Difco)) for 48hrs. The culture was centrifuged at 5000 rpm for 30 min. Filter papers (no.1 Whatman, size;6mm) were saturated by supernatant, then , antibacterial activity of Sa1 was tested against the same four standard strains. (Melconian, *etal.*,1983 and Schmauder,1997).

2-5-2-Primary detection of Staph. aureus1 exotoxin : (Saleh,2000)

Two culture media were used to test the ability of Sa1 to produce exotoxin (having proteolytic activity) : Casein Hydrolysate Agar (CHA) and Skin Milk Agar (SMA) (Oxoid), after inoculation of these media by Sa1 and incubation for 48hrs at 37ºC, a clear zone around the colonies indicating ability of Sa1 to produce a proteolytic enzyme (exotoxin)

2-5-3-Production of Exotoxin : (Taguchi, *etal.,* 1995, and Al-Sarraji,1996)

100 ml of Sa1 was cultured in Casein Hydrolysate Broth (CHB) incubated in shaking incubator in 37ºC at 120 xg / min for 48hrs, then estimated for the biomass of bacterial growth (gm/100ml), clotting activity for crude enzyme solution extracted from supernatant of fermentation culture in (unite/ml), concentration of protein in (mg/ml) and activity in (Unit/mg) .

2-5-3-1-Evalution of clotting activity, modified (Saleh,2000)

Fresh cow milk used to evaluate the clotting activity of Sa1 exotoxin.

1ml of enzyme solution extracted after each stage of purification was added to 10 ml cow milk and incubated in water bath at 35ºC, then rotary movement was carried until clotting indicators were shown.

The clotting activity was measured by the following equation :

$$\text{Milk Clotting Unit (MCU) (Unit / ml)} = \frac{\text{No. mls of crude enzyme solution}}{\text{Clotting time (sec)}} \times 100$$

2-5-3-2-Evalution of protein concentration (Becker,*etal.,*1996)

Lowry assay was used to evaluate protein concentration of crude enzyme solution after each stage of purification according to the standard curve of Bovine Serum Albumin(BSA) (Pharmacia, Sweden) between (0.001-0.01) gm/100ml the protein concentration (mg/ml) was measured by the following equation :

$$\frac{\text{Protein concentration in sample (mg) x inverted dilution}}{1000}$$

2-5-4- Purification of *Staph.aureus* exotoxin :

Three stages of purification were done for *Staph.aureus* exotoxin :

2-5-4-1- precipitation by Ammonium sulphate salt : (Taguchi,*etal.*,1995).

A crude enzyme solution was extracted from CHB , centrifuged at 6000 x g for 20 mins, then 60% w/v (saturation ratio) of $(NH_4)_2\ SO_4$ gradually added until complete dissolving occurred . The solution was left for 1hr at 4ºC, then centrifuged at 12000 xg (rpm/min) for 20min to obtain the precipitated crude exotoxin(enzyme)

2-5-4-2- Membranous Infiltration (Dialysis) technique : (Okino,*etal.,*1996)

150 ml of Tris-HCl buffer (pH = 7.2) was added to 4 gm of precipitated crude exotoxin, dialysis cellulose tubing (Fischer Sci . Co., M.W. Cutoff : 12000-14000) was used at 4ºC for 48hrs, the dialysis solution (distilled water) was changed each 6hrs , until next step this solution was lyophilized.

2-5-4-3- Gel Filtration Chromatography (Kalasz,1984) :

Sephadex G-100 (Pharmacia, Sweden) filled the column (23x2.2cm). 0.01 gm of lyophilized crude exotoxin was dissolved by 0.5 ml of (Tris-HCl buffer, pH=7.2) then added to gel surface, column equipment with buffer solution contineoued in speed (30 ml/hr) , descending solution accumulated as a 5 ml/part by Fraction Collector (LKB,Sweden).

Protein content of pure exotoxin for each part was measured by spectrophotometer (Pyeunicam, SP8-100 , Netherland) at 280 nm, then all parts were united to lyophilize by freeze dryer (Edwards, Pirani,Mod.501,USA) and preserved screw cap bottles for further studies.

2-5-5-characterization of *Staph. aureus* 1 exotoxin

2-5-5-1- lytic activity Sa1 exotoxin (Saleh,2000)

The lytic activity of Sa1 exotoxin was examined by measuring the concentration of L-tyrosine released from lysis substrate (haemoglobin), at 280 nm.
0.1-1 mg/ml of standard L-tyrosine used to make a standard curve..

2-5-5-2- Some Kinetic properties of Sa1 exotoxin

A study of stability of Sa1 exotoxin in various rang of pH (4,7,9) and temperature (30,37,45,60) was evaluated according to Clansky (1990) , by measuring the inhibition zones(mm) of casein (as a substrate)..

2-5-5-3- Cytotoxicity of Sa1 exotoxin (Nair,*etal*.,1989)

Sa1 exotoxin cytotoxicity was tested against human RBCs. 1ml of blood was added to 20ml of sterile normal saline. A serial dilutions of Sa1 exotoxin (0.05-100) µg/ml were dissolved in DMSO (Dimethyl sulfoxide) (BDH , Germany), 100µl of each concentration separately was added to 2ml of blood solution, then left for 1hr to show clearity of blood solution indicated by lysis of RBCs .

2-5-5-4-Antibacterial activity of Sa1 exotoxin (Donham, *etal*.,1988)

(0.1-10) µg/ml of exotoxin were prepared to evaluate the antibacterial activity of Sa1 exotoxin against four of standard strains. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) for the exotoxin were evaluated .

2-5-5-5-Evalution of purity and molecular weight of *Staph aureus* 1 exotoxin by Polyacrylamide Gel Electrophoresis (PAGE) technique

Conventional Polyacrylamide Gel Electrophoresis (PAGE) was used to evaluate the purity of Sa1 exotoxin and to measure the molecular weight by horizontal gel electrophoresis (LKB,mod. 2117 multiphore ,Sweden)

Preparation of solutions:

1. Tris –Glycin buffer stock solution (0.15 M, pH: 8.9)
2. Electrode buffer solution O .
3. Polyacrylamide gel solution F (7.5 %) W/V .
4. Ammonium persulphate solution G (15mg/ml) .
5. TEMED (Tetramethyl Ethylene Diamine) solution.
6. Bromophenol blue solution (0.25% W:V)
7. A standard protein solution : the following standard proteins were supplied by (BDH, Germany) to make standard curve : Trypsin Inhibitor (21000 Dalton (D)), RNA

polymerase (39000D), Bovine Serum Albumin (BSA) (67000 D), Aldolase (140000 D), Catalase (232000 D), Ferritin (440000D), and Thyroglobulin (669000 D).

8. A stock exotoxin solution and purified exotoxin ranged between (0.1 to 2 mg/ml).10 µl of bromophenol blue solution was added to each 250 µl of exotoxin.

Procedure

Pre electrophoresis was carried out according to standard instructions supplied by LKB(Application Note 306) with a current of 50 mA , Voltage of 15v/cm for 30 mins ,and 10 µl of samples were added with a current 20mA, 15V/cm for 10min then the current adjusted to 40mA for 3hrs (as far as the bromophenol blue come near the gel ending).

Molecular weight of purified Sa1 exotoxin was calculated by drew the relation between log. of molecular weight for standard proteins and its relative mobility (Rm) by the following equation :

$$Rm = \frac{\text{Distance of protein mobility until bands shown}}{\text{Distance of bromophenol blue until the current end}}$$

2-6-Extraction of bacterial antigens

2-6-1- All bacterial antigens (Antigenic extracts)

antigenic extracts of each bacterial type isolated from AD lesion was carried according to a procedure proposed by (Saikh & Bhattacharyya, 1984), with some modifications to fit our experiments :

1. 18-20 hrs culture for each bacteria were suspended by PBS , washing and centrifugation at 3000xg for 30 mins, and removing the supernatant were carried .
2. 5ml pf PBS was added to precipitate, then mixing by rotary mixer (vortex,Germany) at 2000xg for 15mins.
3. Each antigenic suspension separately sonicated by (Ultrasonic disintegrator, MSE, Soniprep 150, U.K) at 15 microns/sec for 20 mins under cooling condition .
4. The suspension was centrifuged at 10000 xg for 30 mins in 5ºC, then three times repeated washing by PBS and centrifugation at 300xg for 30mins.
5. Supernatant lyophilized by freeze dryer (Edwards, Pirani, Mod. 501,USA), and preserved by screw cap bottles until use.

2-6-2-*Staph.aureus*1 outer membrane proteins (OMPs) (surface antigens)

Isolation of *Staph.aureus*1 OMPs was carried according to a procedure proposed by (Ortiz, *etal*.,1989), with some modification to fit our experiments.

1. *Staph.aureus*1 was cultured in Brain-Heart Infusion , and incubated in shaking incubator for 18hrs in 37ºC at 80 rpm/min(xg).
2. The suspension was centrifuged at 3000 rpm/min for 30mins, the supernatant would remove , and the precipitate was washed by 0.01 M of HEPES (N-2-Hydroxy Ethyl Piprazin- N- Ethane Sulfonic acid) with Triton X-100 .
3. Three times repeated step-2.
4. Mixed the suspension by rotary mixer (Vortex) at 2000 xg for 15mins.
5. Sonication, centrifugation, and lyophilization of the suspension were same as previous procedure(2-6-1).

2-7- *In Vivo* study

To evidence the germ theory and to document any suggestions that interest in relation between atopic dermatitis and their possible bacterial causative agents , so, the in vivo study was found necessary and useful . However, we proposed these investigation to get these evidence ,also, to identify the possibility of experimentally induce of atopic dermatitis (human being origin) in animal model , and to determine the similarity between human AD and mice AD .

The laboratory animals was the white mice (*Mus musculus*) BALB/C, aged between 14-16days, weighted 25-30 gm grew under standard conditions.

The mice were divided into groups as follow :

1. Infected by Intradermal injection.
2. Infected by spot technique (kept infectious agents on externally determinant area).
3. Infected by Prick technique (streaking the epidermal layers).
4. Control group without any infections

Each above mentioned groups divided into subgroups and each one infected separately by :

1. pure extracted *Staph.aureus*1 exotoxin .
2. *Staph aureus*1 OMPs.
3. *Staph aureus* all body antigens.
4. Viable cells of *Staph. aureus*1 in two various doses
   I. 25 Sa1 cell /0.1ml.
   II. 50 Sa1 cell /0.1ml.

The pathogenicity and clinical complications of mice skin were monitored in serial period times .

2-8- Histopathological study

An oval shaped, 10mm diameter pieces of skin of patients , treated mice and control were examined histopathologically. Fixed in 10% formalin 6mm sections stained by hematoxylin- Eosin stain according to (Elenitsas & Halpern, 1997 ;WHO,2002 and 2003) to reveal the histopathological changes (by an expert of histopathologists).

2-9-Statistical Analysis

In order to determine the statistical significances among different variables, SPSS program (Statistical Program for Social Sciences) ver.11 , and Minitab program ver.10 (to find statistical between variables) were used for these purposes .

The following statistical testes were performed :

Chi-square (χ^2) test, univariate and multivariate logistic regression analysis, the ANOVA analysis was applied for correlation between each study parameters, and the differences between two proportions by T-test were used to assess the significance of difference between groups. P-Value less than 0.05 was considered as statistically significant (S),P-value <0.01 as highly significant (HS), and P-value <0.001 as extremely significant (ES).

2-10- Statistical measurement of Sampling size

Minimum sampling size of AD patient was measured by : (PCMDI, 1998, and NHQM,2004) :

$$\text{(MSS)} \quad n = \frac{no}{1 + 1/N\,(no-1)} \qquad \text{while } no = \frac{Z^2PQ}{d^2}$$

no : Essential primary number
Z : critical value at p<0.05 (1.96)
P : known proportion ratio (20%)
Q : Failure rate (1-P)
d : Acceptable error (0.05)

N : population size

2-11-Standard bacterial strains

The following standard bacterial strains were used in this investigation

Bacterial types		supplied source
Staph.aureus	**ATCC 25923**	Epidemiol lab. CHL, Baghdad
Staph.aureus	**NCTC 6571**	Biotechnol lab. Coll. Science. Basrah
Staph.epidermidis	**ATCC 12228**	Epidermiol lab.,CHL
Strept. faecalis	**ATCC 19433**	Epidermiol lab.,CHL
E.coli	**ATCC 25922**	Epidermiol lab.,CHL
E.coli	**NCTC 5933**	Biotechnol lab.
Ps. aeruginosa	**ATCC 27853**	Epidermiol lab.,CHL
Ps. aeruginosa	**NCTC 6750**	Biotechnol lab.
Kl. Pneumonia	**ATCC 10031**	Biotechnol lab.
B. subtilis	**PCI 219**	Biotechnol lab.

Chapter three
The results

3-1- Epidemiological (Demographical) and clinical studies

A total of 484 patients with atopic dermatitis , 211 (43.6%) males and 273(56.4%) females, with a control group of 100 healthy persons (without any allergic , immunologic, or skin infection disorders) were enrolled in this study .

3-1-1- Age groups and Sex (Figures 1 & 2)

The age of patients with atopic dermatitis (AD) ranged from 1 to 50 years with a statistical mean of age of (11.42) years, patients of age groups (infantile & childhood) recorded a higher prevalence of AD(34.7%) & 27.5% respectively) than adulthood patients (groups 3,4,5) that recorded (17.8%, 11.4% and 8.7% respectively) (P< 0.001) .

Males were highly affected with AD in groups (1,4,5) (55.36%, 43.28% and 32.84% respectively), while females predominated males in groups (2 & 3), (61.65% and 60.35% respectively) (P<0.001) .

3-1-2- Disease locations (sites of predilection) Fig (3)

Eight locations were predominantly affected in patients with AD included in this study. In general we can statistically arrange all sites of predilection of AD lesions according to high percentage as follow : Face (33.1%) > Face & neck (20%) > All body (13%) > upper & lower limbs (11.4%) > Face & upper limbs (9.1%) > Face & upper & lower limbs(7.9%) > upper limbs (5.6%) .

The Face & neck were the predominant site of AD lesions in males (94 cases) while in females , Face was the predominant site (111 cases) (P<0.001)

In other view, according to the age, the percentages of the predominant site of AD lesions in age groups (1,2,3,4 and 5) were (face 77% , face & upper limbs 100% , face & upper & lower limbs 100% , upper limbs and upper & lower limbs each in 53% , and upper & lower limbs 40% respectively) (P<0.05) .

3-1-3- Personal history (associated disorders) (Fig.4)

The study showed that AD were significantly associated with various disorders , particularly other atopic diseases (allergic sinusitis , bronchial asthma , allergy , fungous infections , tuberculosis , pneumonia , pharyngitis , otitis media and urinary tract infections which has been reported in (145 (68.7%) and 197(72.1%))of the cases of male and female respectively . On other hand , no associated disorder was reported in (66(31.2%) and 76(27.8%)) of the cases in males and females respectively . (P<0.001).

3-1-4-. Family history (Fig.5)

occurrence rates of atopic disorders among families of AD patients were found very highly significant (P<0.001) . AD, AS and BA were recorded in the following manner (42M: 42F , 50M :64F , and 42M : 56F) cases respectively, while a total of 188 cases (77M : 111F) showed no family history of atopy .

3-1-5- Skin types (Fig. 6 & 7)

Amongst the skin types recorded in AD patients , dry skin was the major one; 289(59.7)(109M : 180F) , followed by seborrheic and natural types of skin (45M:78F and 57M:15F respectively).

A very highly significant differences have been shown between distribution of skin types of AD patients according to age groups (P<0.001) , and there are a highly statistically correlation between skin types and prevalence of AD (appendix -1-) .

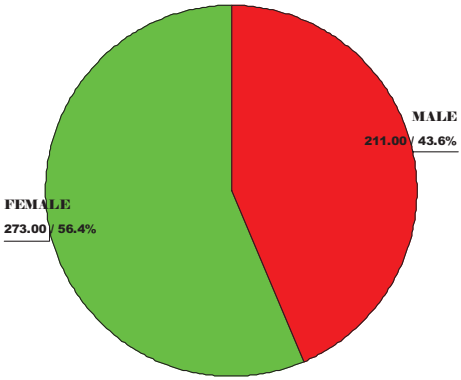
The dry skin was more predominant in the first three age groups followed by seborrheic skin, while natural skin was more predominant in 4th and 5th age groups (P<0.001)

3-1-6- Disease Course (Fig.8)

The sub acute pattern of AD presented highly in all age groups except for the first age group , where chronic pattern is predominantly reported followed by acute pattern (P<0.001) .

3-1-7- Severity (Fig.9)

Mild degree was predominant in the first group, while sever degree was predominant in the second age group. Moderate degree was commonest among patients in other age groups. (P<0.001)



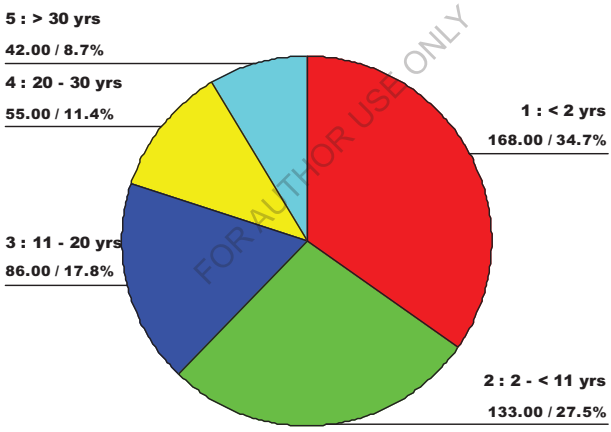


FIG- 1 - : DISTRIBUTION OF AD CASES FOR BOTH SEXES (ABOVE) ACCORDING TO AGE GROUPS (BELOW). (P < 0.001)

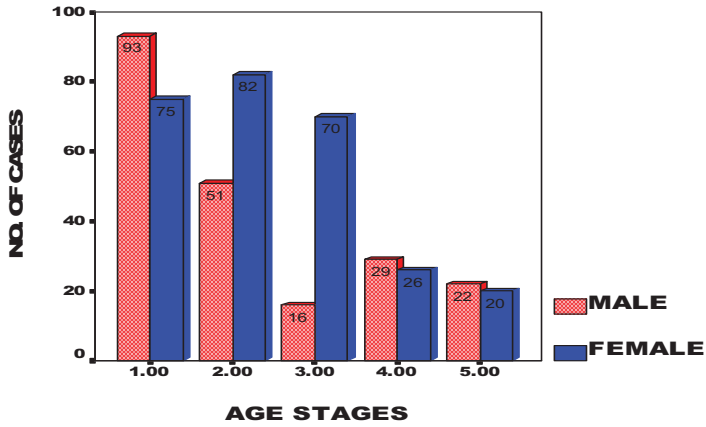


FIG - 2 - : SEX DISTRIBUTION AMONG AD PATIENTS IN VARIOUS AGE GROUPS . (P< 0.001)

FIG - 3 - : SITES OF PREDILECTION OF AD LESIONS . (P < 0.001)

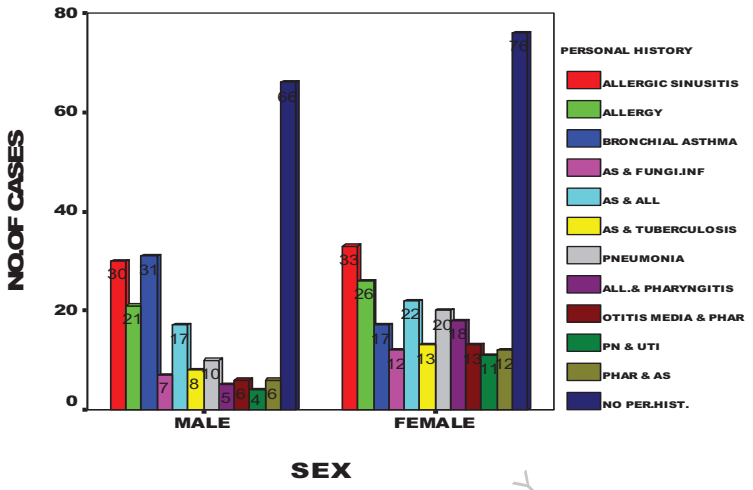


FIG - 4 - : ASSOCIATED DISORDERS IN AD PATIENTS . (P < 0.001)

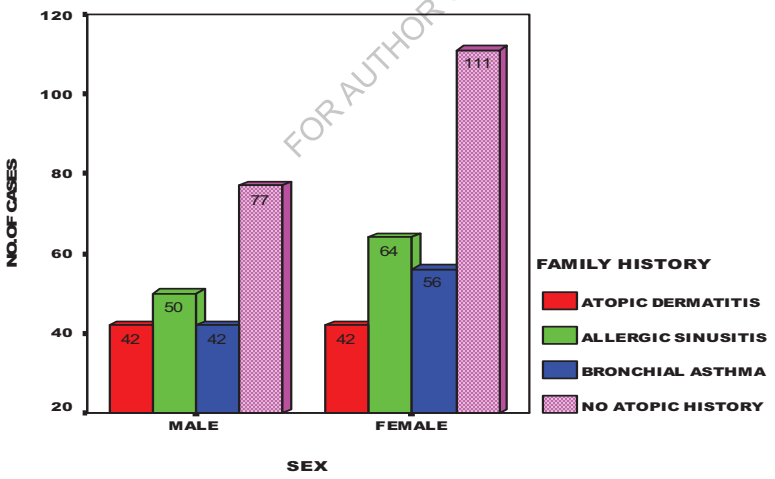


FIG - 5 - : OCCURANCE RATES OF ATOPIC DISORDERS AMONG FAMILIES OF AD PATIENTS . (P < 0.001)

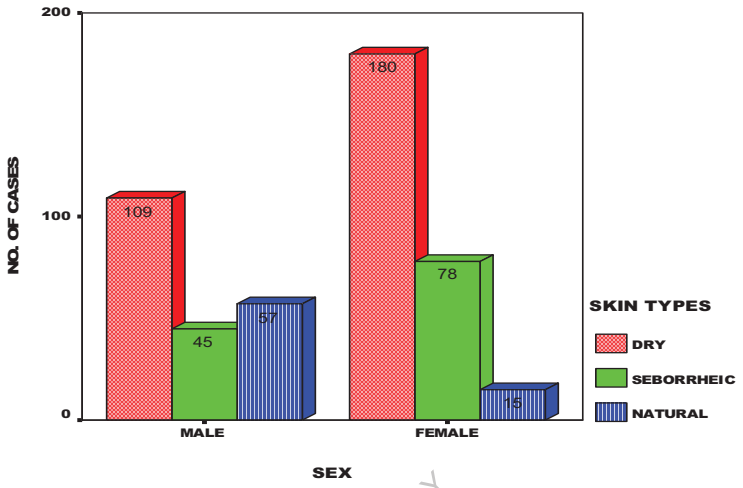


FIG - 6 -: SKIN TYPES OF AD PATIENTS FOR BOTH SEXES . (P < 0.001)

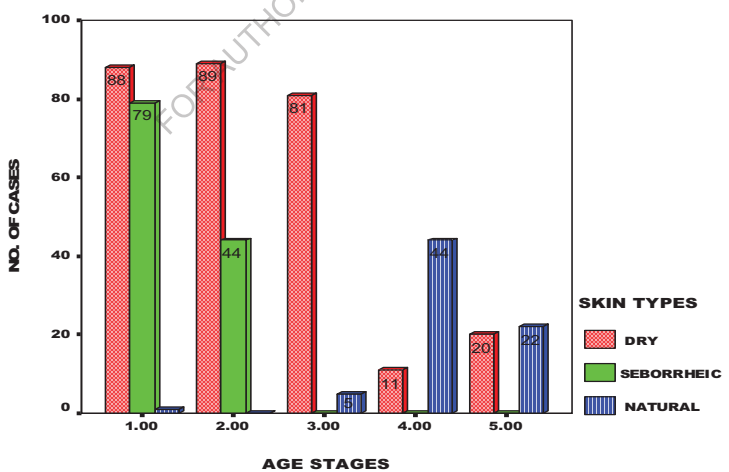


FIG - 7 -: DISTRIBUTION OF SKIN TYPES OF AD PATIENTS ACCORDING TO AGE GROUPS . (P < 0.001)

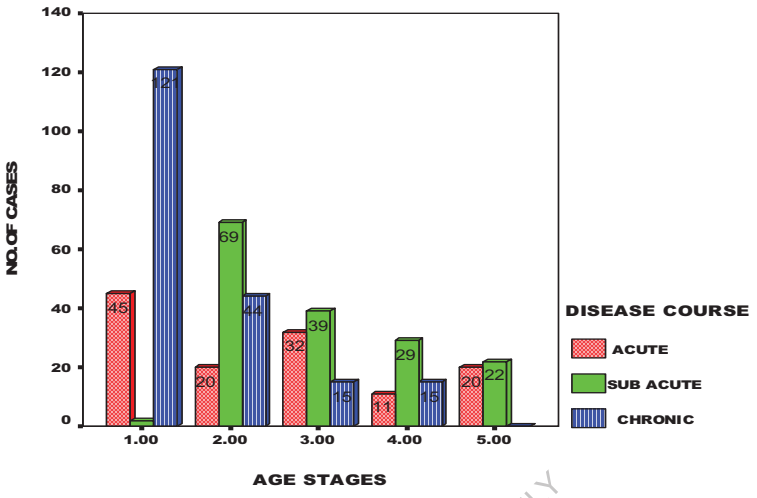


FIG - 8 -: THE PATTERNS OF AD PRESENTATION IN VARIOUS AGE GROUPS . (P < 0.001)

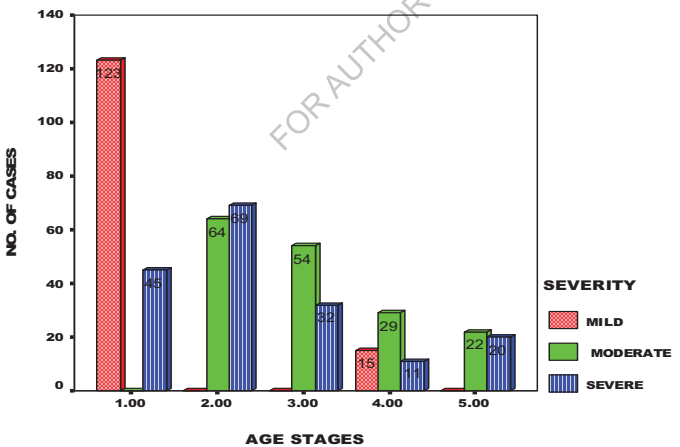


FIG - 9 -: DEGREE OF SEVERITY FOR AD PATIENTS ACCORDING TO AGE GROUPS . (P < 0.001)

3-2- Haematological study

Uni & multivariate logistic regression analysis of various blood components or parameters was done to illustrated statistical relationships between blood components and affection with AD (Age , Sex , and other epidemiological and clinical parameters) and within each of these blood components.

The study showed that all above associations were statistically very highly significant ($P<0.001$).

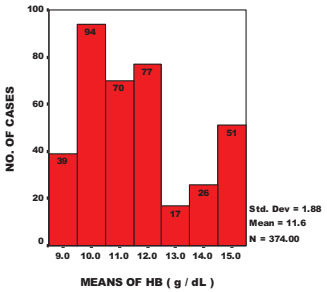
All results of haematological study illustrated in **figures (10)** . Which can be summarized as follow according to comparison with standard and /or control values. Values of HB ,RBCs count, neutrophils, monocytes and basophiles : were under the normal or control values in (54.3%, 67.6%, 7.8%,7.5% and 70.6%) of AD patients respectively , while the values of ESR, W.B.Cs count, Lymphocytes%, lymphocytes count, Eosinophils, Basophiles and platelets count were above the normal or control values in (41.2%,50.8%,4.0%,62.3%,76.7%,20.3%, and 51.6%) of AD patients respectively.

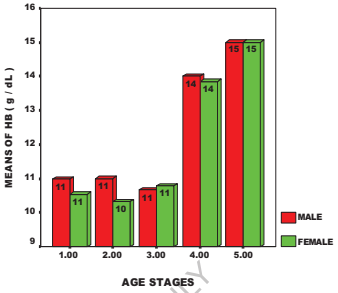
It is important to notice that values of Neutrophils, Lymphocytes and monocytes were approximately near the control value, so, there is no statistically significant differences in the values ($P\geq0.05$) .

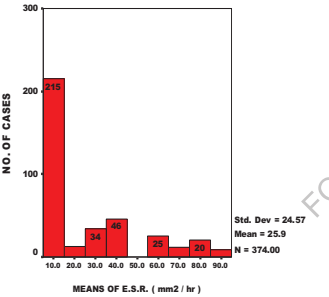
Statistical similarities between mean values of blood components illustrated in **figure (18) ,** showed that platelets , eosinophils, W.B.Cs count, were strongly correlated with each others in similarities ranged between 85 - 92%, while others have a different levels of similarities ($P<0.001$).

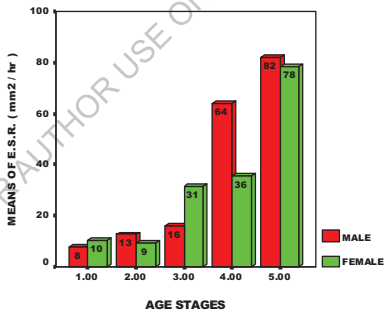
FIGs - 10 - : ILLUSTRATED ALL OF MEASURED BLOOD COMPONENTS FOR AD PATIENTS. (P < 0.001).

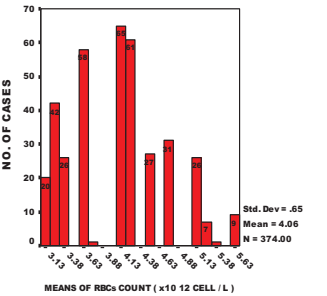
RIGTH: STATISTICAL HAEMATOLOGICAL MEANS FOR ALL AD PATIENTS.
LEFT: DESECRIPTIVE HAEMATOLOGICAL MEANS FOR VARIOUS AGE GROUPS OF AD PATIENTS (MALE & FEMALE) .

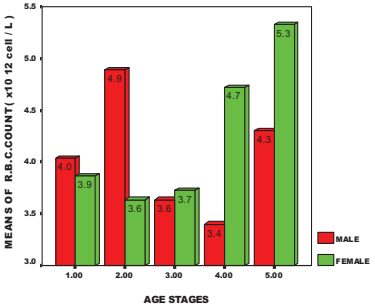


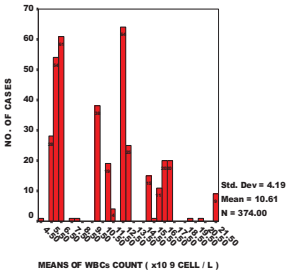


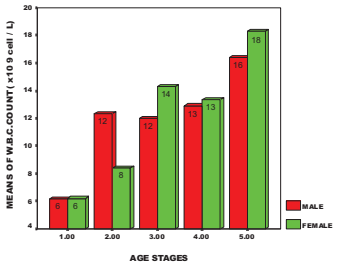


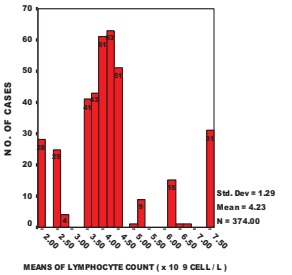


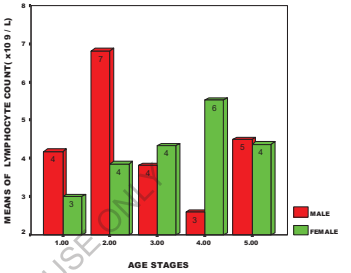


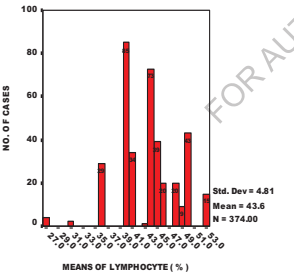


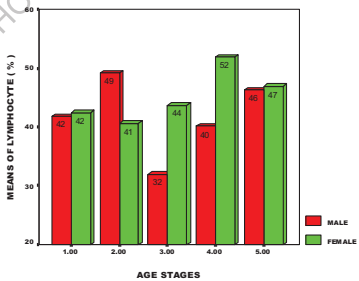


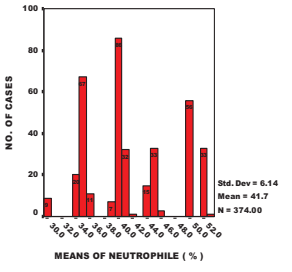


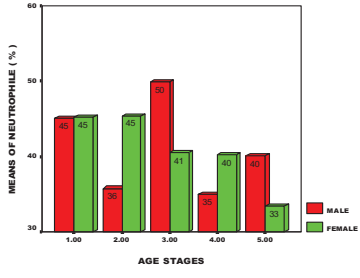


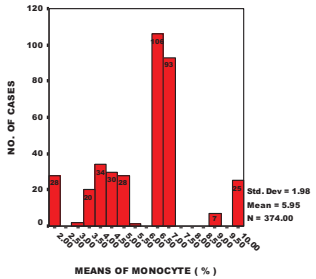


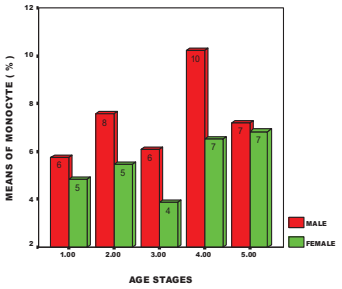


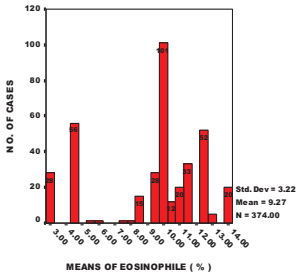


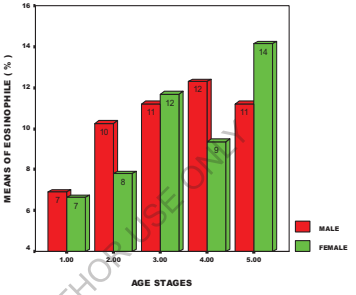


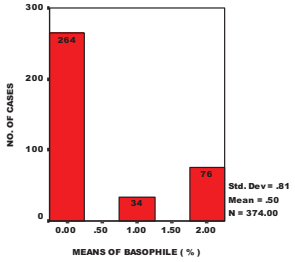


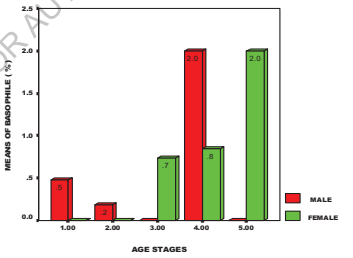


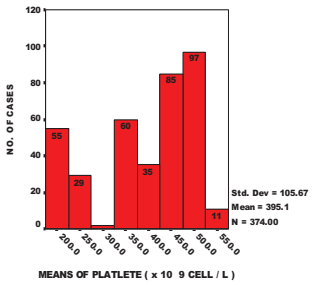


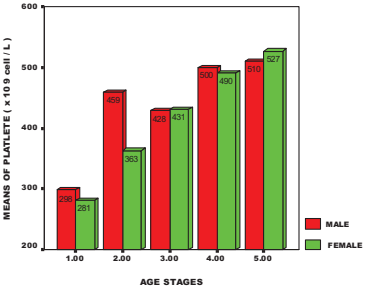












3-3- Immunological study

3-3-1- Clusters differentiation (CD)

All values of CD (CD_3 , CD_4 , CD_8 and CD_{19}) were elevated above normal or control means in (65.7%,75.9%,94.4% and 68.5%) from AD patients respectively with a significant differences($P<0.05$).**figures (11)** .

The similarity analysis of CD_s **(Figure 17)** illustrated that CD_3, CD_4 , and CD_8 have a similarity ranged between 94-97%, while in contrast , CD_{19} showed a similarity ratio 63.86% .

3-3-2- Immunoglobulins and components of complement (IgA, IgG, IgM, total IgE, C3 and C4)

All mean values of IgA, IgG, IgM, C_3 and C_4 were increased in comparison with mean values of normal or control groups in (68.9% , 67.5% , 67.5% , 69.3% and 78.3%) of AD patients respectively with a very high statistically significant differences ($P<0.001$) **figure(11)**.

The total concentration of IgE was also elevated above the normal value in about 86.5% of AD patients in all age groups .($P<0.001$). **figure (11)** .

The modes of allergy of AD patients in various age groups were studied and illustrated in figures (11). It has been found that the mode of allergy , very probable (IgE>100 IU) , is predominant in (88.8%) of various age groups of AD patients ($P<0.001$).

From the study of similarity analysis, it has been found that IgA ,IgG,IgM,C_3 and C_4 were strongly correlated in similarity ranged from 94-95.5% ,while IgE have similarity with other in value of 79.79% ($P<0.001$). **Fig. (16)**

3-3-3- Specific IgE-Allergens

Figures (12) illustrated the types of sensitivity of AD patients according to allergens- specific IgE reaction measured by EIA technique.

From 40 environmental (food ,fungal ,agricultural ,chemical and aero) - allergens, it has been recognized that allergens which induced highest degree of reactions (highest type of hypersensitivity (B , A , H and above)) for AD patients in order of frequency were as follow : ($P<0.001$) .

G_1(86.5%) , W_{28}(80.5%) , U_{82}(77.0%) , F_{29} , (75.8%) , F_3(74.7%) , U_{81}(70.2%) , G_7(64.6) , W_{15} (62.8%) , F_2(61.8%) , E_{85}(42.2%) , F_{108}(42.1%) , E_1(41.6%) , E_7(41.0%) , U_{73}(35.4%) , F_{83}(35.3%) , T_{204}(33.7%) , T_{11}(33.1%) , E_5(30.9%) , F_{75}(29.8%) ,I_6(29.6%) , F_{47}(29.2%) , G_{15}(27.0%) , M_5(24.7%) , T_9(24.5%) , C_1(23.6%) , E_3(23.1%) , F_1(22.5%) , E_4(19.6%) , and F_{81}(17.4%) .

Also it have been found that there is no significant differences for the following allergens to induce hypersensitivity $P \geq 0.05$: U_{83} , F_9 , F_{12} ,F_{15} , F_{24} , F_{25} , F_{35} , M_1 , M_2 , M_3 , and M_{19} .

From 11 extracted bacterial antigens (as allergens) it has been recognized that these allergens , same as previous (according to highest modes), were as follow: ($P < 0.001$) Sa1. enzy (100%), Sa3 All (95.5%) , Se All(84.3%) , Sa1 All (82.6%) ,

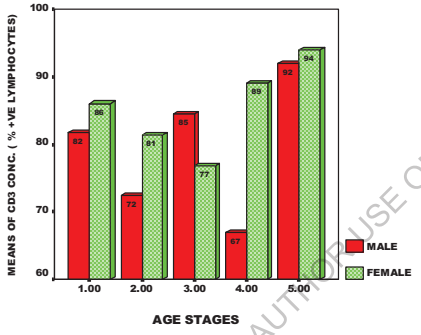
St fec All (71.9%) , St pyo All (69.1%) , pr ac All (68.0%) , *E coli* All (63.5%) , Sa1 body (52.2%) , and Pseu All (50.0%) .

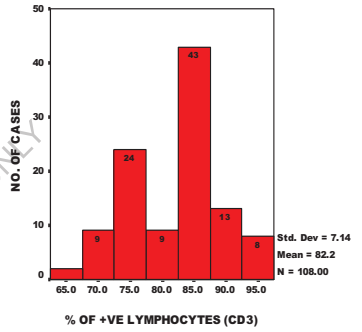
According to the results of statistical similarity analysis illustrated in **fig. (15)** , it has been found that various degree of correlation were reported between all studied allergens for the environmental allergens , the degrees of similarity ranged from (75.64 – 98.25%), while for the extracted bacterial allergens the similarity ranged from (73.30 – 91.36%) ($P<0.001$) .

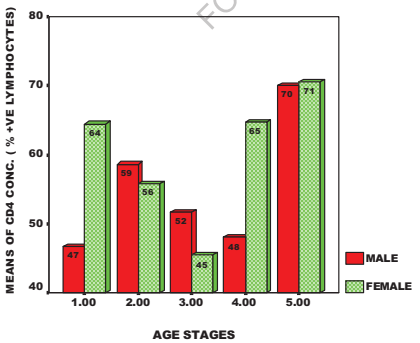
FIG - 11 -: **ILLUSTRATED ALL OF STUDIED IMMUNOLOGICAL PARAMETERS FOR AD PATIENTS. (P < 0.001)**

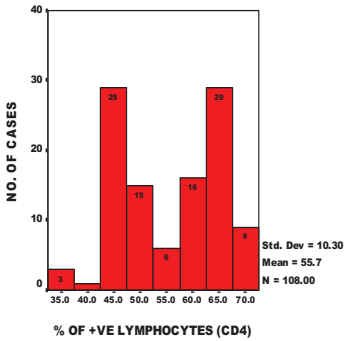
IMMUNOLOGICAL PARAMETERS :
CLUSTERS OF DESIGNATION (CD): CD3 , CD4 , CD8 AND CD19 .
COMPLEMENTS (C) : C3 AND C4 .
IMMUNOGLOBULINS (Ig) : IgA , IgM , IgG , and total IgE .

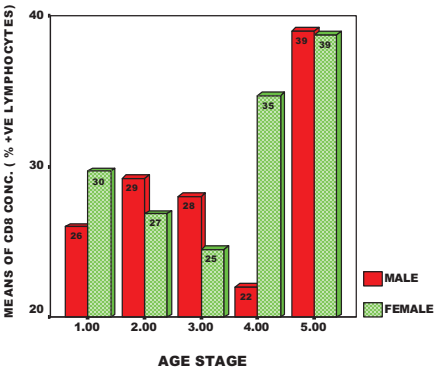
LEFT : **STATISTICAL IMMUNOLOGICAL MEANS FOR ALL AD PATIENTS .**
RIGTH **: DESCRIPTIVE IMMUNOLOGICAL MEANS FOR VARIOUS AGE GROUPS OF AD PATIENTS (MALE & FEMALE) .**

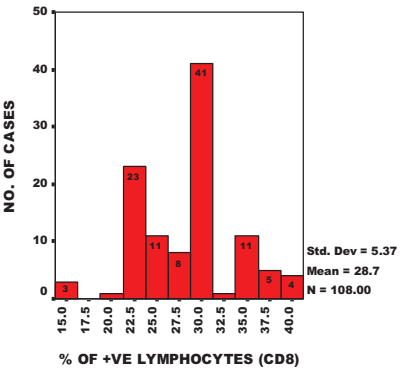


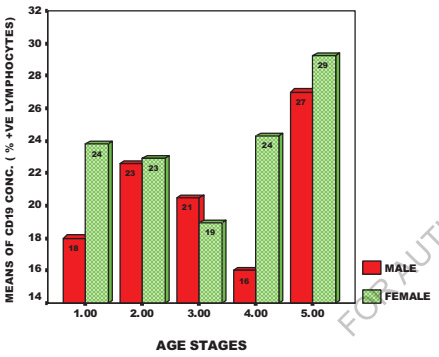


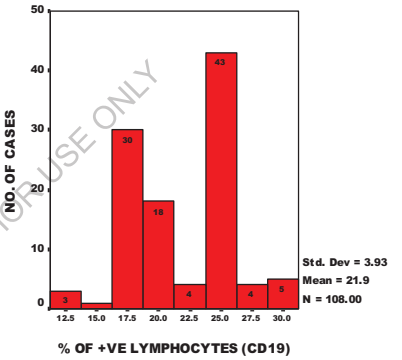


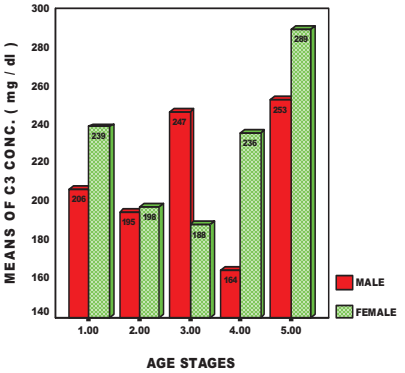


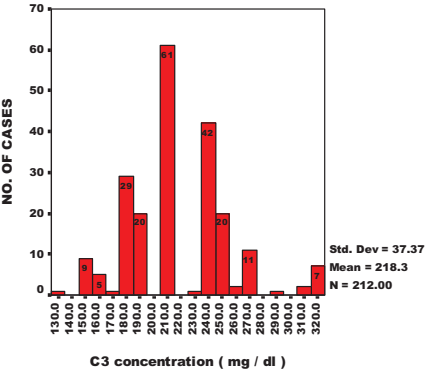


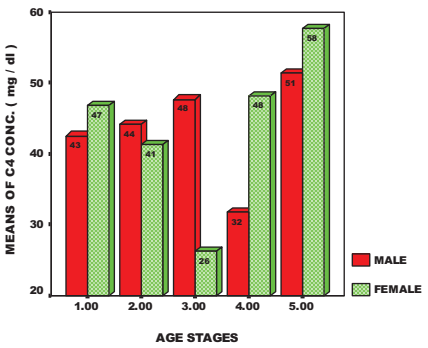


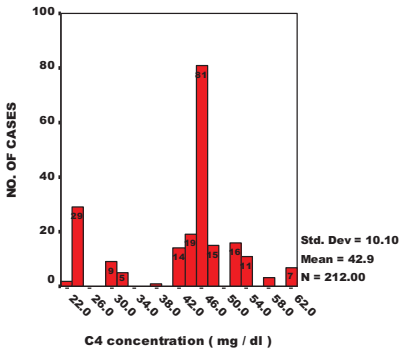


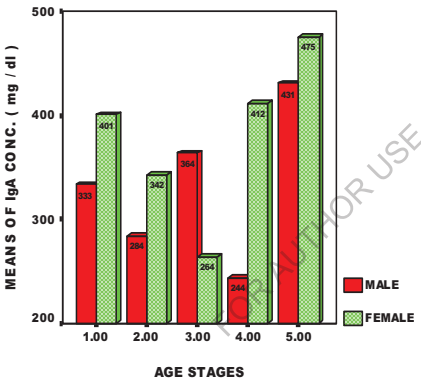


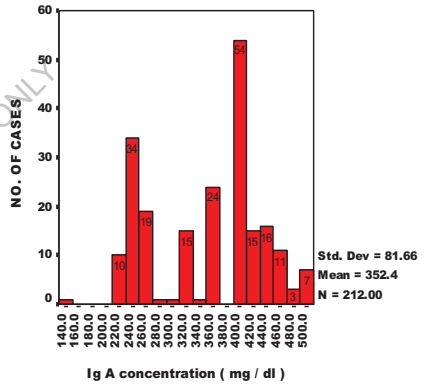


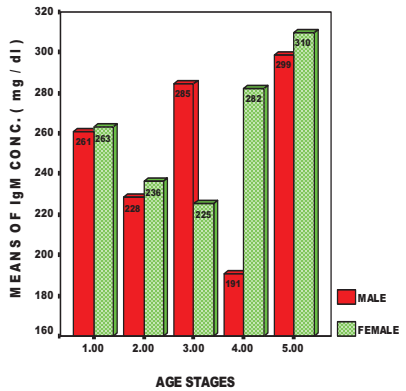


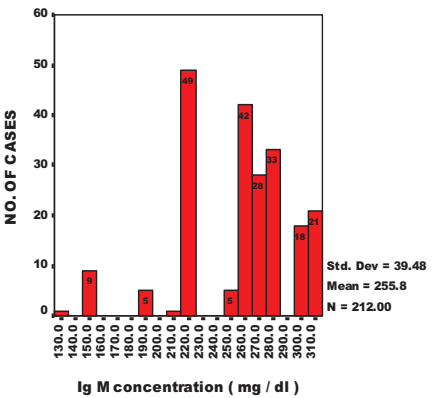


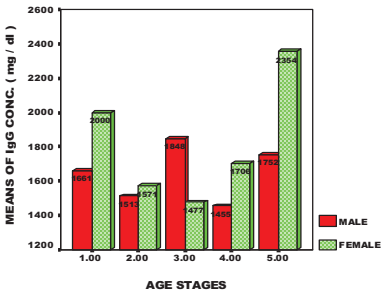


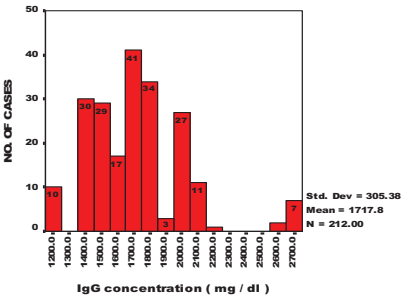


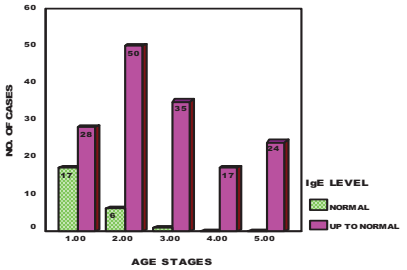


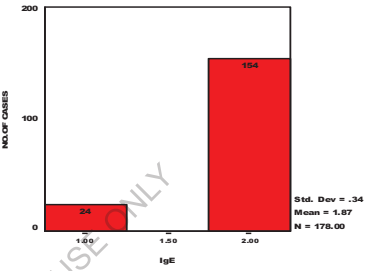


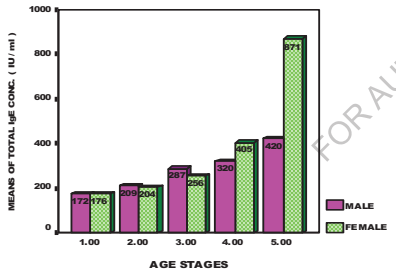


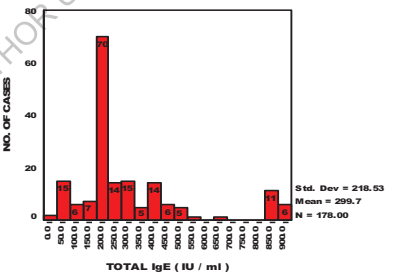


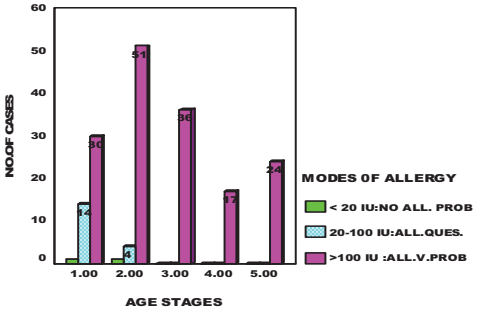


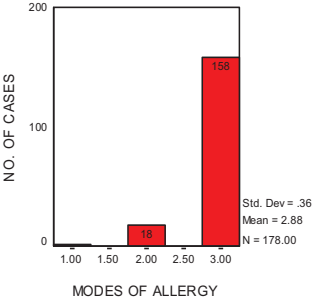












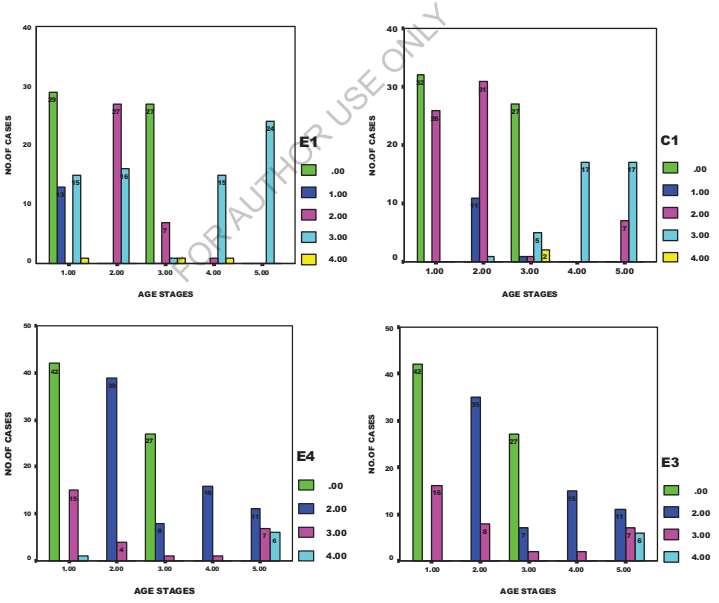
FIGs - 12 - : ILLUSTRATED ALL OF ALLERGENS – SPECIFIC IgE REACTIONS (TYPES OF SENSETIVITY) (DETRMINATION OF ALLERGENS – IgE LEVELS) MEASURED BY USE AN ENZYME IMMUNOASSAY (EIA) FOR AD PATIENTS (WITH ALLERGENS EXTRACTED FROM BACTERIAL ISOLATES) . (P < 0.001) .

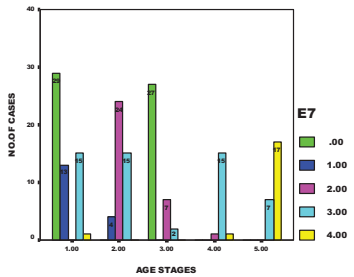
COMMON ALLERGENS

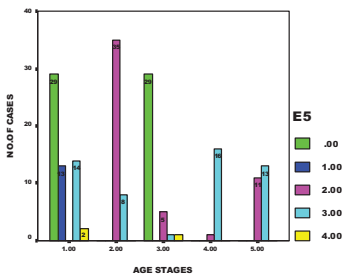
C1 : PENCILLOYL G , E1 : CAT EPITHELIUM , E3 : HORSE EPITHELIUM , E4 : COW DANDER , E5 : DOG DANDER , E7 : PIGEON DROPPINGS , E75 : CHICKEN FEATHERS , F1 : EGG WHITE , F2 : MILK , F3 : CODFISH , F9 : RICE , F12 : PEA , F15 : WHITE BEAN , F24 : SHRIMP , F25 : TOMATO , F29 : BANANA , F35 : POTATO , F47 : GARLIC , F75 : YOLK , F81 : CHEDDAR CHEESE , F83 : CHICKEN , F108 : ONION , G1 : SWEET VERNAL GRASS , G7 : COMMON REED , G15 : WHEAT , I6 : COCKROACH (GERMAN) , M1 : *PENCILLIUM NOTATUM* , M2 : *CLADOSPORIUM HERBARUM* , M3 : *ASPERGILLUS FUMIGATUS* , M5 : *CANDIDA ALBICANS* , M19 : *ASPERGILLUS VERSICOLOR* , T9 : OLIVE , T11 : PLANE TREE , T204 : PEPPER TREE (*SCHINUS MOLLE*) , U73 : SILK , U81 : COTTON CULTIVATED , U82 : SHEEP`S WOOL , U83 : FORMALDEHYDE , W15 : SCALE , W28 : ROSE .

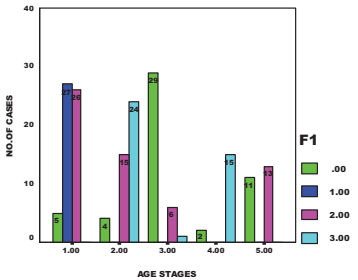
EXTRACTED BACTERIAL ALLERGENS

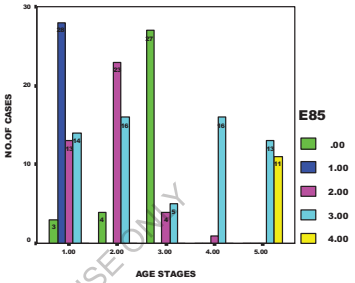
SAI.ENZY : *STAPH. AUREUS* 1 (EXOTOXINE) ,SAI.ALL : *STAPH AUREUS*1 (ALLERGEN), SA1.BODY : *STAPH. AYREUS* 1 (EXTRACTED BODY) , SA2.ALL : *STAPH. AYREUS* 2 (ALLERGEN) , SA3.ALL : *STAPH. AYREUS* 3 (ALLERGEN) , SE.ALL : *STAPH. EPIDERMIDIS* (ALLERGEN) , STFE.ALL : *STREP. FAECALIS* (ALLERGEN),STPY.ALL : *STREP. PYOGENES* (ALLERGEN) , PR.AC.ALL : *PROBIONIBACTERIUM ACNES* (ALLERGEN) , PSEU.ALL : *PSEUDOMONAS* (ALLERGEN),E.COLI.ALL : *ESCHERICHIA COLI* (ALLERGEN)

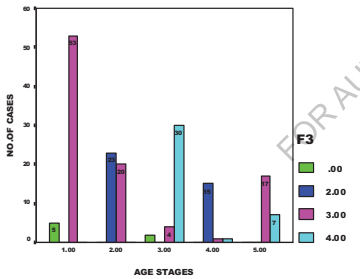


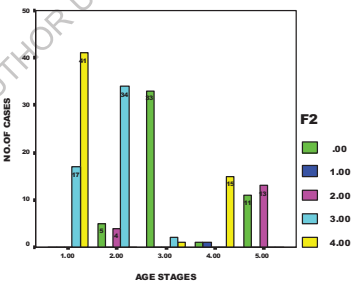


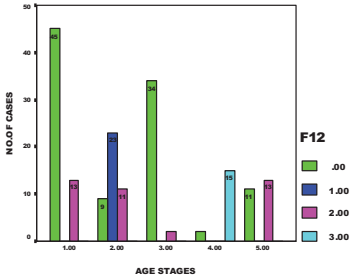


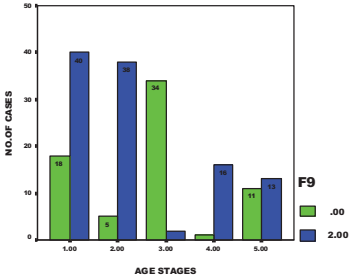


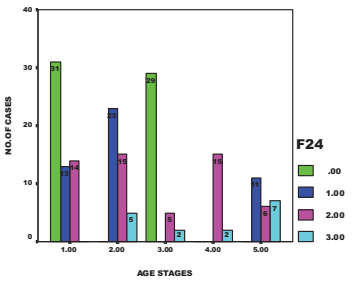


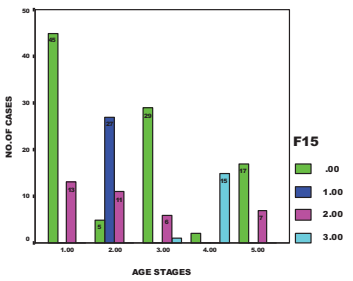


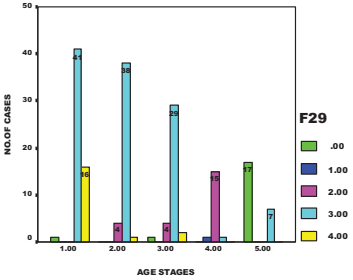


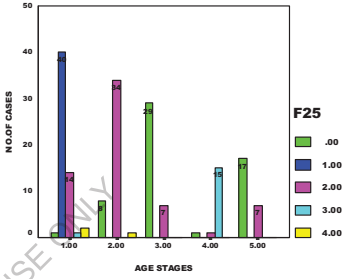


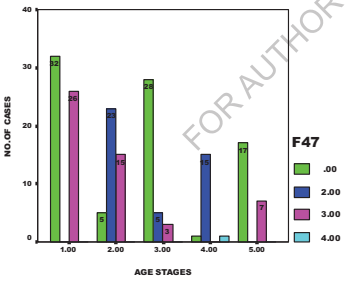


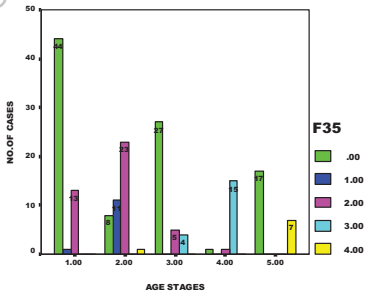


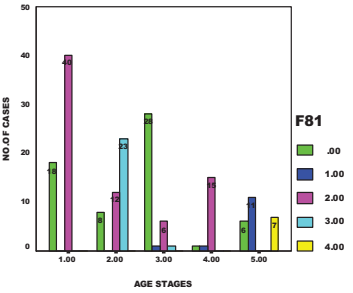


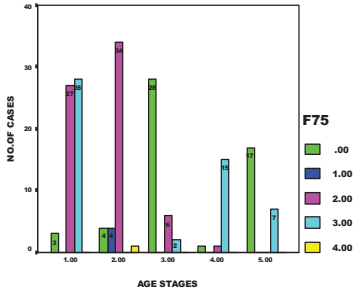


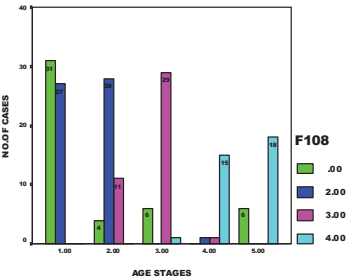


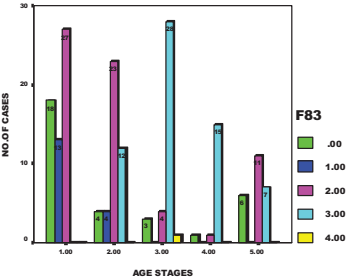


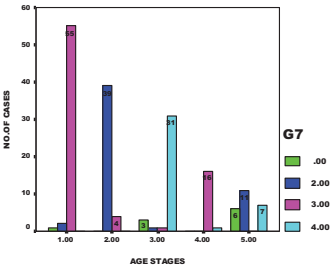


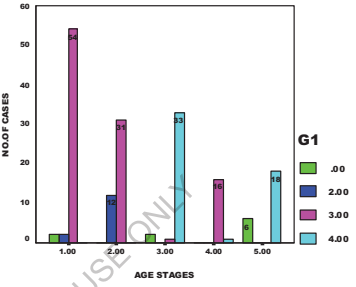


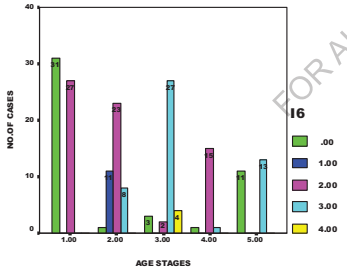


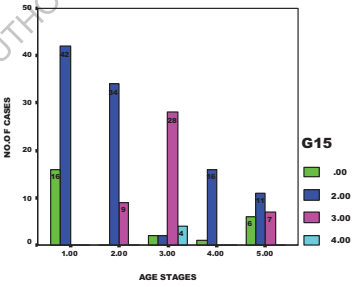


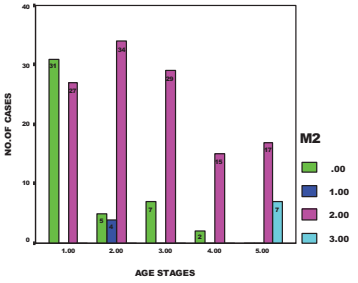


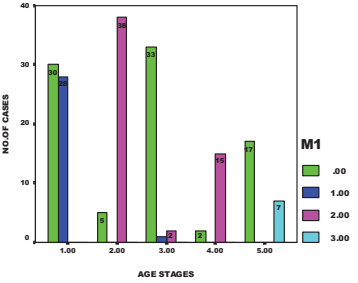


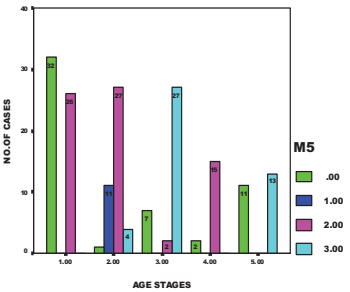


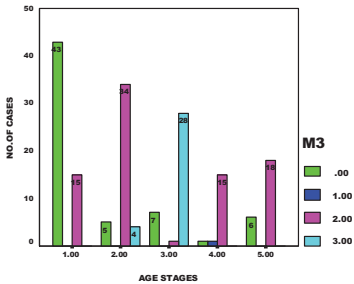


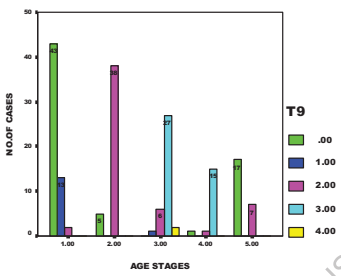


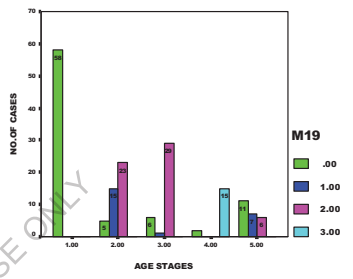


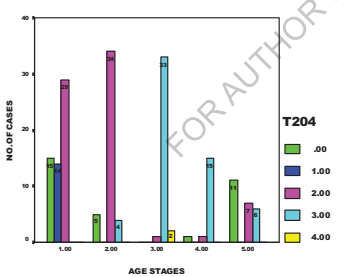


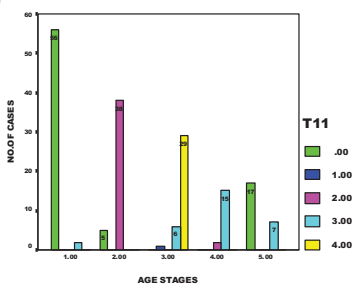


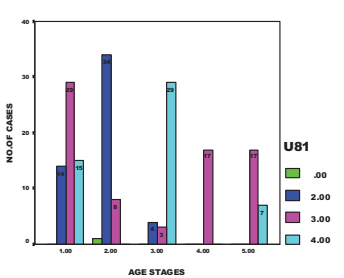


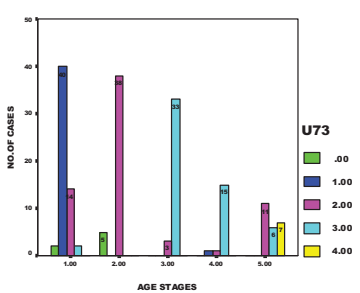


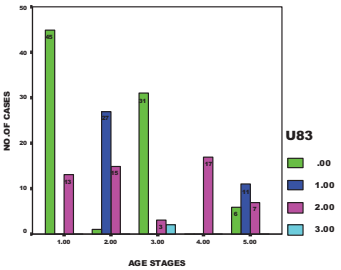


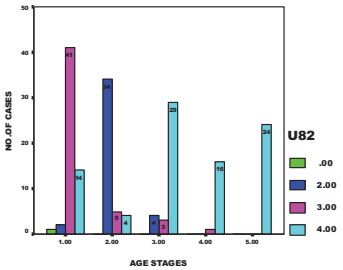


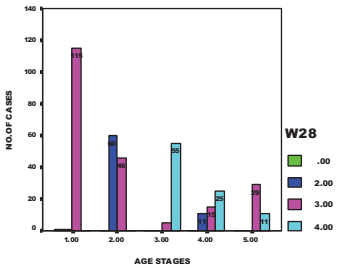


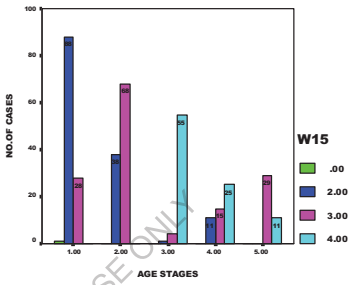


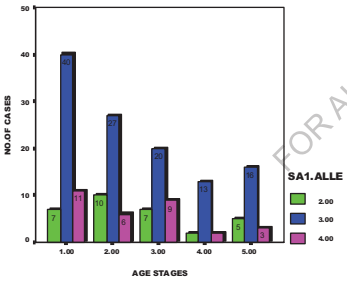


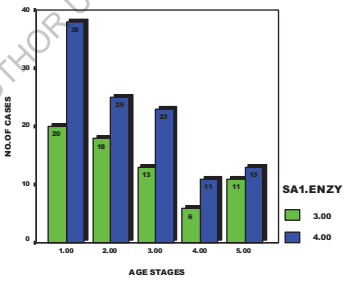


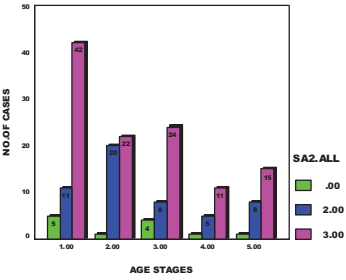


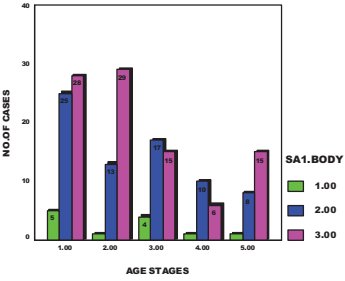


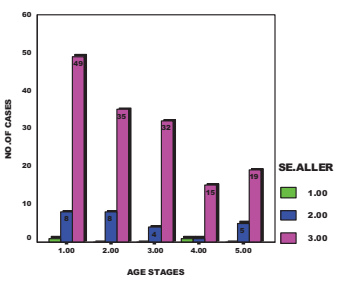


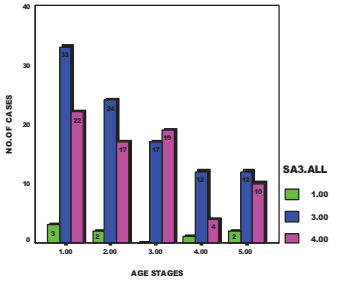


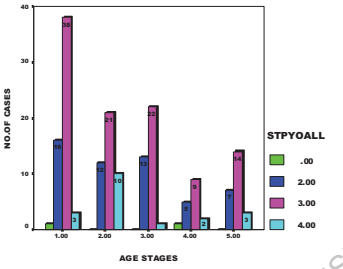


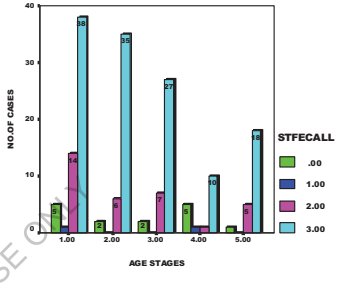


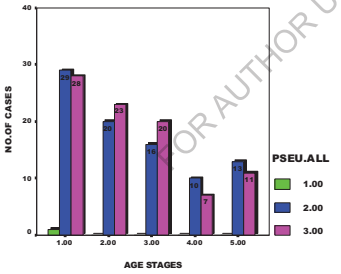


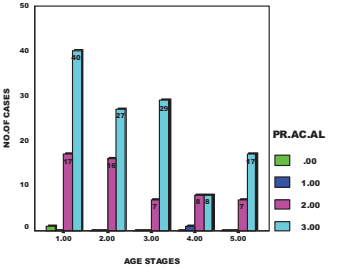


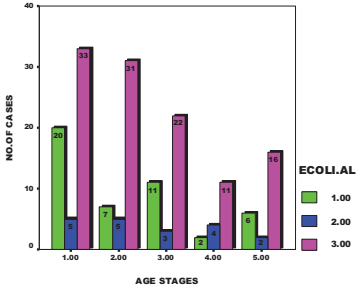












3-4- Bacteriological Study

3-4-1- Bacterial types and numbers

Table (1) illustrated all bacterial types isolated from eczematous lesions and healthy skin . The total number of positive culture was(270(94.4%)) out of 286 of eczematous lesions and (247(86.36%) of the healthy sites. In general , twenty bacterial types were isolated from both areas separately and (959 , 744) isolates with isolation ratio (3.35:1 , 2.6:1) isolates : case were identified in each of above area respectively . The percentages of bacterial occurrence in eczematous lesions and healthy skin respectively were as follow : ($P<0.05$) .
Staph. aureus (60.48% , 17.48%) , *Staph. epidermidis* (17.13% , 57.34%) , *Staph. .xylosus* (2.79% in each) , *Staph. saprophyticus* (5.24% , 10.48%) , *Staph. capitis* (2.79% in each) , *Staph. hominis* (22.37%, 9.44%) , *Strept. pyogenes* (17.13% , 9.79%) , *Strept. faecalis* (23.07% , 17.83%) *Strept. mutans* (14.68% , 9.44%) , *E.coli* (25.52% , 33.21%) *Enterobacter* sp. (5.59% , 17.83%) , *Klebsiella* sp. (3.14% , 1.39%) , *Acinetobacter* sp. (5.59% , 3.49%) *Proteus* sp. (5.94% in each) , *Pseudomonas aeruginosa* (17.48% , 5.59%) , *Probionibacterium acnes* (19.58% , 3.49%) *Pr.granulosum* (20.27% , 18.53%) *Haemophilus influenzae* (21.32% , 11.53%) , *Bacteroid* sp. (18.18% , 3.84%) , and *Corynebacterium* sp. (26.92% , 17.83%) .

Table (2) illustrated modes of isolation of bacterial types from eczematous lesions and healthy skin of AD patients. It has been found that the two bacterial agents that were predominant in each eczematous lesions and healthy skin in percentages (44.11% , 41.29%) respectively followed by other modes , with a statistically significant differences between modes of isolation ($P<0.05$) .

Table (1) : Bacterial types isolated from eczematous lesions and healthy skin of AD patients. (P<0.05)

Bacterial types	No. of cases(%) from Eczematous lesion	No. of cases(%) from healthy skin
Staph.aureus	173(60.48)	50(17.48)
Staph.epidermidis	49(17.13)	164(57.34)
Staph. xylosus	8(2.79)	8(2.79)
Staph. saprophyticus	15(5.24)	30(10.48)
Staph. capitis	8(2.79)	8(2.79)
Staph. hominis	64(22.37)	27(9.44)
Strept.pyogenes	49(17.13)	28(9.79)
Strept. faecalis	66(23.07)	51(17.83)
Strept. Mutans	42(14.68)	27(9.44)
E.coli	73(25.52)	95(33.21)
Enterobacter sp.	16 (5.59)	51(17.83)
Klebsiella sp.	9(3.14)	4(1.39)
Acinetobacter sp.	16(5.59)	10(3.49)
Proteus sp.	17(5.94)	17(5.94)
Ps. Aeruginosa	50(17.48)	16(5.59)
Pr. Acnes	56(19.58)	10(3.49)
Pr. granulosum	58(20.27)	53(18.53)
H. influenzae	61(21.32)	33(11.53)
Bacteroid sp.	52(18.18)	11(3.84)
Corynebacterium sp.	77(26.92)	51(17.83)
No. of isolates	959	744
Average (isolate :case)	(3.35: 1)	(2.6 : 1)
No. of –ve growth culture	16(5.59)	39(13.63)
No. of +ve growth culture	270(94.4)	247(86.36)
Total No. of cases	286	

Table(2) : Modes of isolation of bacterial types from eczematous lesions and healthy skin of AD patients (P<0.05)

Mode of isolation	No. of cases (%) from eczematous lesions	No. of cases (%) from healthy skin
Single	38(14.07)	31(12.55)
Double	75(44.11)	102(41.29)
Third	22(12.94)	19(7.69)
Fourth	19(11.17)	61(24.69)
Fifth	59(34.7)	17(6.88)
Sixth and over	57(33.52)	17(6.88)
No. of +ve culture (total No. of modes)	270 (94.4)	247(86.36)
No. of -ve culture	16(5.59)	39(13.63)
(total No. of cases)	286	286

Figure (13) showed the distribution of bacterial types in Eczematous lesions and healthy skin of AD patients .

The means of bacterial number measured for eczematous lesions and healthy skin of AD patients showed in **figures (14)** . The bacterial numbers ranged from (0.02 – 92.0) x 10^5 cell/cm and (0.11-23.0) x 10^3 cell/cm for each of the above mentioned sites respectively , with a very statistically high significant differences between means of bacterial numbers in the same area and between both studied sites . ($P<0.001$) .

3-4-2- Antibiotics susceptibility

Table(3) illustrated antibiotic susceptibility patterns of *Staph aureus* isolated from eczematous lesions and healthy skin against various antibiotics .

The percentages of sensitivity mode against antibiotics in each of the above mentioned areas are respectively as follows :
Amoxycillin / Clavulanic acid (Ac) (87.28% , 66.0%) , Bacitracin (B) (86.7% , 72.0%) , Clindamycin (Cd) (53.75% , 70.0%) , Cephalothin (Ch) (70.52% , 62.0%) , Co-trimoxazole (Co) (24.85% , 46.0%) , Doxycyclin hydrochloride (Do) (31.21% , 50.0%) , Chloramphenicol (C) (48.55% , 64.0%) , Erythromycin (E) (17.91% , 10.0%) Gentamicin (G) (73.41% , 100%) , Methicillin (M) (46.82% , 72.0%) , Rifampicin (R) (74.56% , 78.0%) , Tetracyclin (T) (49.13% , 80.0%) , and Vancomycin (Va) (89.59% , 100%) .

A high statistically significant differences ($P<0.01$) have been found between a three modes of antibiotic susceptibility within the same antibiotics and between these modes of various antibiotics.

To determine the modes of antibiotics resistance (how many antibiotics are resisted by *Staph. aureus* ??). **Table (4)** shows the percentages of antibiotic resistance modes of *Staph.aureus* according to the largest percentages as follows : ($P<0.05$)
(36.41% , 24.27% , 16.76% , 13.87% and 8.67%) of resistance to three , double , single , four , and five or more of antibiotics respectively for eczematous lesions , in contrast to (36.0% , 22.0% , 20.0% , 14.0% , and 8.0%) of resistance to double , single , three , four and five or more of antibiotics respectively for healthy skin .

The statistical similarities between antibiotic affecting modes on *Staph. aureus* showed in **figure (19)** . It has been found that the antibiotic affecting *Staph.aureus* isolated from eczematous lesions are strongly correlated with each other in similarity ranged from (88.75% – 99.13%) and splitted from the same antibiotics affecting *Staph.aureus* isolated from healthy skin that also closely related with each others and have a similarity ranged from (97.10% -100%) ($P<0.001$) .

Table - 3 -:Antibiotics susceptibility patterns of Staph. aureus isolated from eczematous lesions (D),and healthy skin (N) against various antibiotics. (P < 0.01)

ANTIBIOTICS	SITE	RESISTANCE	INTERMEDIATE	SENSITIVE
AC	D	13 (7.5)	9 (5.2)	151 (87.28)
	N	17 (34.0)	-	33 (66.0)
B	D	11 (6.35)	12 (6.9)	150 (86.7)
	N	9 (18.0)	5 (10.0)	36 (72.0)
CD	D	27 (15.6)	53 (30.63)	93 (53.75)
	N	5 (10.0)	10 (20.0)	35 (70.0)
CH	D	16 (9.24)	35 (20.23)	122 (70.52)
	N	8 (16.0)	11 (22.0)	31 (62.0)
CO	D	104(60.11)	26 (15.02)	43 (24.85)
	N	15 (30.0)	12 (24.0)	23 (46.0)
DO	D	102 (58.9)	17 (9.82)	54 (31.21)
	N	21 (12.13)	4 (8.0)	25 (50.0)
C	D	53 (30.63)	36 (20.8)	84 (48.55)
	N	-	18 (36.0)	32 (64.0)
E	D	116 (67.05)	26 (15.02)	31 (17.91)
	N	22 (44.0)	23 (46.0)	5 (10.0)
G	D	-	46 (26.58)	127 (73.41)
	N	-	-	50 (100.0)
M	D	50 (28.9)	42 (24.27)	81 (46.82)
	N		14 (28.0)	36 (72.0)
R	D	25 (14.45)	19 (10.98)	129 (74.56)
	N	4 (8.0)	7 (14.0)	39 (78.0)
T	D	75 (43.35)	13 (7.51)	85 (49.13)
	N	10 (20.0)	-	40 (80.0)
VA	D	5 (2.89)	13 (7.51)	155 (89.59)
	N	-	-	50 (100.0)
TOTAL NUMBER OF CASES (286)				
NO. OF POSITIVE CULTURES OF *STAPH AUREUS* FROM ECZEMATOUS LESIONS (D) : 173 (60.48 %)				
NO. OF POSITIVE CULTURES OF *STAPH AUREUS* FROM NORMAL SKIN (N) : 50 (17.48 %)				
NO. OF NEGATIVE CULTURES OF *STAPH AUREUS* FROM ECZEMATOUS LESIONS (D) : 97 (33.9 %)				
NO. OF NEGATIVE CULTURES OF *STAPH AUREUS* FROM NORMAL SKIN (N) : 197 (68.88 %)				
NO. OF TOTAL POSITIVE CULTURES FROM ECZEMATOUS LESIONS (D) : 270 (94.4 %)				
NO. OF TOTAL POSITIVE CULTURES FROM NORMAL SKIN (N) : 247 (86.36 %)				
NO. OF TOTAL NEGATIVE CULTURES FROM ECZEMATOUS LESIONS (D) : 16 (5.59 %)				
NO. OF TOTAL NEGATIVE CULTURES FROM NORMAL SKIN(N) : 36 (13.63 %)				

Table(4) : illustrated modes of Antibiotic resistance of *Staph.aureus* isolated from Eczematous lesions and Normal skin. (P<0.05)

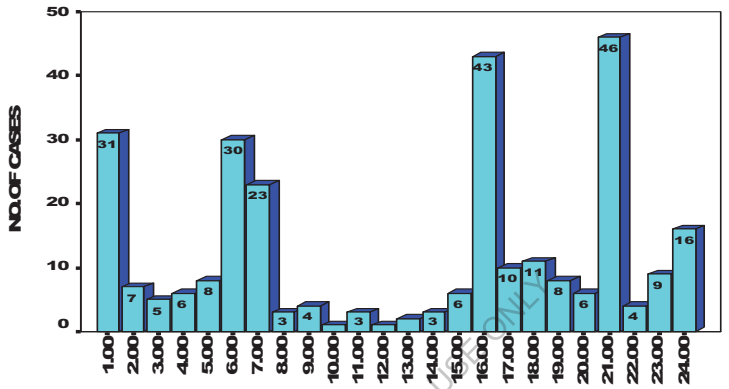
Mode of antibiotic resistance	No. of isolates from Eczematous lesions(%)	No. of isolates from healthy skin %
Single Antibiotic	29(16.76)	11(22.0)
Double antibiotic	42(24.27)	18(36.0)
Three antibiotic	63 (36.41)	10 (20.0)
Four antibiotic	24 (13.87)	7(14.0)
Five or more antibiotic	15(8.67)	4(8.0)
No. of Staph.aureus isolates	173	50

FIGURE (13) : ILLUSTRATED ISOLATION MODES OF BACTERIAL TYPES FROM ECZEMATOUS LESIONS (ABOVE) & HEALTHY AREA (BELOW) OF AD PATIENTS SKIN . (P< 0.001)

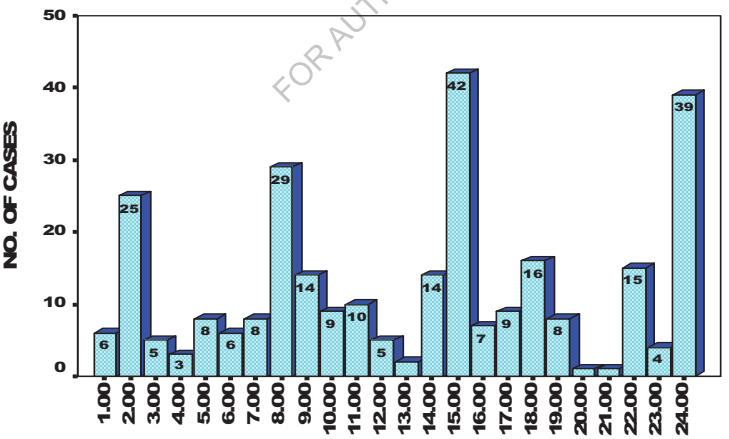
BACTERIAL TYPES : 1 :*STAPH AUREUS* , 2 : *STAPH EPIDERMIDIS* , 3: *STAPH XYLOSUS* , 4 : *STAPH SAPROPHYTICUS* , 5 : *STAPH CAPITIS* , 6 : *STAPH HOMINIS* , 7 : *STREPT PYOGENES* , 8 : *STREPT FAECALIS* , 9 : *STREPT MUTANS* , 10: *E.COLI* , 11 : *ENTEROBACTER SP.* , 12 : *KLEBSIELLA SP.* , 13 : *ACINETOBACTER SP.* , 14: *PROTEUS SP.* , 15: *PS AERUGINOSA* , 16: *PR ACNES* , 17: *PR GRANULOSUM* , 18: *H INFLUENZAE* , 19: *BACTEROID SP.* , 20 : *CORYNEBACTERIUM SP.*

ISOLATION MODES : (AS SHOW IN FIGURES) DEPEND ON ABOVE NUMBERS

1 : 1 , 2: 2 , 3 : 1&2 , 4 : 1&2&7 , 5 : 1&2&8 , 6: 1&7 , 7 : 1&9 , 8 : 2&8 , 9 : 2&4 , 10 : 2&6 , 11 : 2&7 , 2: 2&8 , 13 : 2&9 , 14: 2&10 , 15: 2&10&11&17 , 16: 1&10&17&19&20 , 17: 7&11&13&16&20 , 18 : 4&6&9&10&14&18&20 19 : 3&5&8 , 20: 6&9&10&13&14 , 21: 1&6&8&15&16&18 , 22: 10&15&18&20 , 23 : 12&17&19&20 , 24: NO GROWTH .

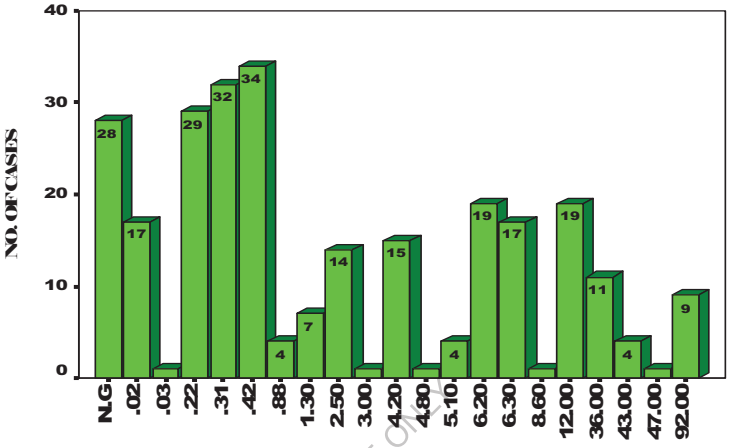


BACTERIAL TYPES ISOLATED FROM ECZEMATOUS LESION

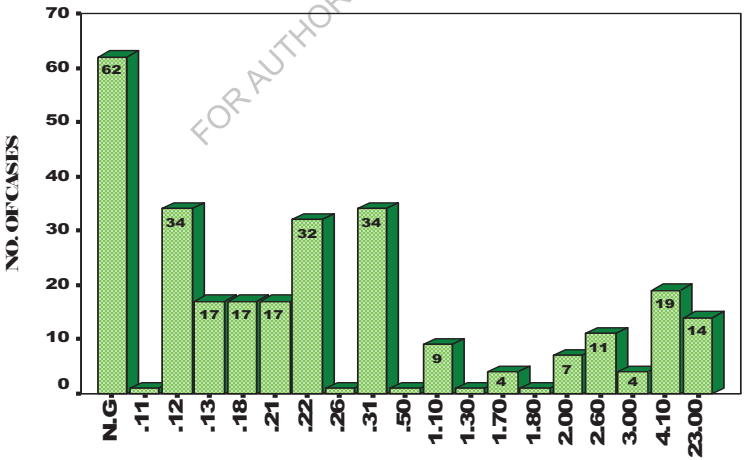


BACTERIAL TYPES ISOLATED FROM HEALTHY AREA

FIGURE (14) : ILLUSRATED MEANS OF BACTERIAL NUMBERS MEASURED FOR ECZEMATOUS LESION AND HEALTHY AREA OF AD PATIENTS . (P < 0.001)



MEANS OF BACTERIAL NUMBERS (x 10 5 CELL / cm) OF ECZEMATOUS LESIONS



MEANS OF BACTERIAL NUMBERS (x 10 3 CELLS / cm) OF HEALTHY AREA

FIGURE (15) : STATISTICAL SIMILARITIES BETWEEN ALL STUDIED IgE - SPECIFIC ALLERGENS . (P< 0.001)

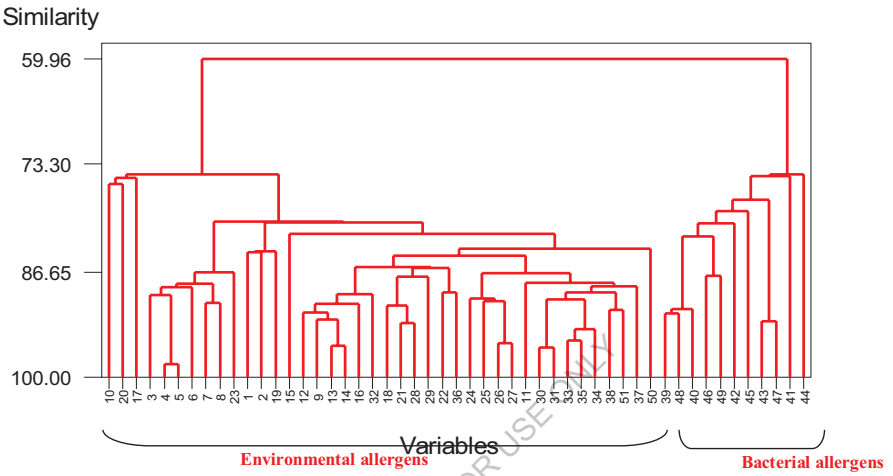


FIGURE (16) : STATISTICAL SIMILARITIES BETWEEN VARIOUS TYPES OF IMMUNOGLOBULINS AND COMPLEMENTES(P< 0.001)

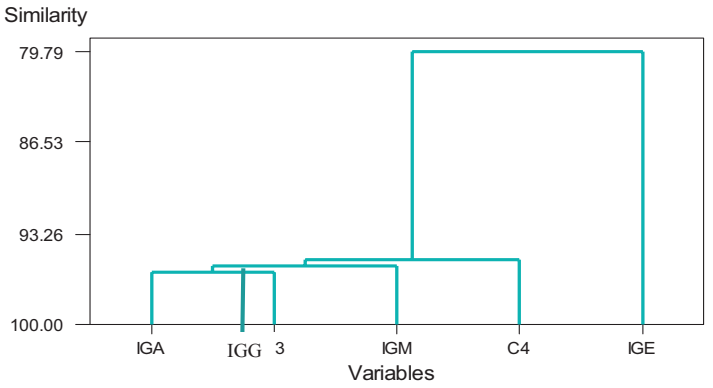


FIGURE (17) : STATISTICAL SIMILARITIES BETWEEN VARIOUS TYPES OF CLUSTERS OF DIFFERENTIATION (CD) .(P< 0.001)

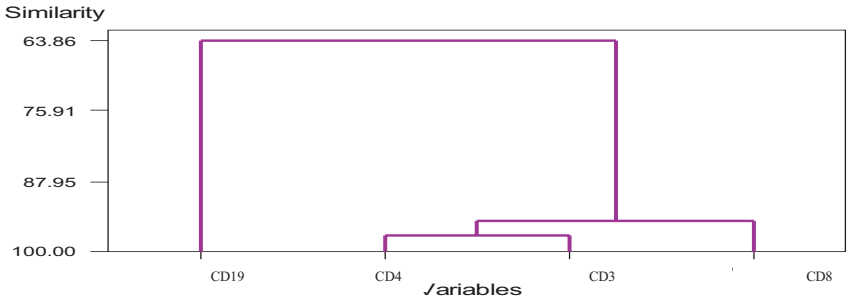


FIGURE (18) : STATISTICAL SIMILARITIES BETWEEN MEANS OF BLOOD COMPONENTS OF AD PATIENTS . (P< 0.001).

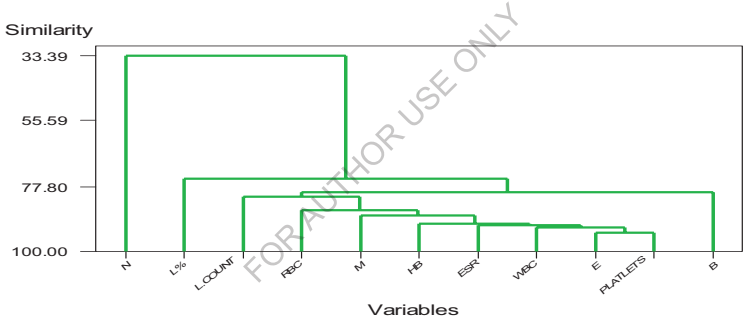
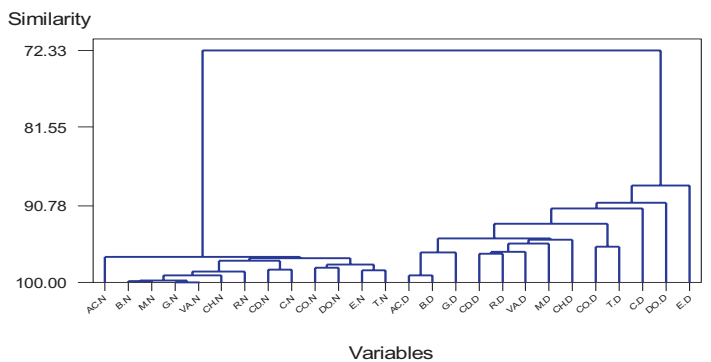


FIGURE (19) : STATISTICAL SIMILARITIES BETWEEN ANTIBIOTICS AFFECTING MODE ON *STAPH . AUREUS* ISOLATED FROM ECZEMATOUS (D) AND HEALTHY (N) AREA OF AD PATIENTS . (P < 0.001)



3-5-Extraction , purification, Identification and Characterization of *Staph.aureus* exotoxin(Superantigen)

3-5-1- Primary screening

Table (5) : illustrate diameters of inhibition zone of five isolates of the most common bacteria isolated from eczematous lesions (*Staph.aureus*) against a standard bacterial strains .

It has been found that *Staph.aureus* 1 (Sa1) was more active than others according to diameter of the inhibition zones (8, 6 , 7.5 , and 10) mm against *E.coli*, *Staph.aureus*, *B.subtilis* and *K.pneumoniae* respectively ($P<0.05$) .

Table(5) : Primary screening of *Staph.aureus* strains isolated from eczematous lesions of AD patients against a standard bacterial strains ($P<0.05$)

Staph.aureus strains	Diameter of inhibition zones (mm)			
	E.coli NCTC 5933	*Staph.aureus* NCTC 6571	*B.subtilis* PCI 219	*K.pneumoniae* ATCC 10031
Sa1	8	6	7.5	10
Sa2	4	2	3.5	5
Sa3	3.5	1	3	2.5
Sa4	1.5	0	2	1.5
Sa5	3	1.5	2.5	3.5

Antibacterial activity of Sa1, after incubation , against same above mentioned standard bacterial strains gave the following inhibition zones : (18,20)mm , (7.5,8)mm , (20,23)mm and (25,27.5)mm in 24 and 48hrs after incubation respectively without a significant differences between various period times ($P\geq0.05$). **Table(6)**.

Table(6) : Antibacterial activity of Sa1 against a standard bacterial strains after various incubation periods ($P\geq0.05$)

Incubation period (hrs)	Diameter of inhibition zones (mm)			
	E.coli NCTC 5933	*Staph.aureus* NCTC 6571	*B.subtilis* PCI 219	*K.pneumoniae* ATCC 10031
24	18	7.5	20	25
48	20	8	23	27.5

3-5-2- Primary detection of Sa1 exotoxin

Table(7) : illustrates a primary detection of Sa1 exotoxin on two media , the growth on CHA was heavy and gave 17 mm of inhibition zone , while a slow growth detected on SMA with a diameter of 13mm of inhibition zone.

Medium	Inhibition zone (mm)	Growth
CHA	**17**	+++
SMA	**13**	+

Table(7) : primary detection of Sa1 exotoxin on two culture media .

3-5-3- production of Sa1 exotoxin

The growth of Sa1 on Casein Hydrolysate agar (CHA) gave a biomass (11g/100ml), and specific activity (7.21 unit/mg) while the growth on skim milk agar (SMA) gave a biomass (4g/100ml) and a specific activity (5unit/mg).

According to all previous results that have been mentioned , it has been found that fermentation medium (CHA)(growth and production media) was the best medium used to produce exotoxin from *Staph.aureus*1 , so, the CHA used in all future studies .

3-5-4- Purification of Sa1 exotoxin

Table(8) illustrates the result of the purification steps of Sa1 exotoxin:

3-5-4-1- Precipitation by $((NH_4)_2SO_4)$ Ammonium sulphate salt

Ammonium sulphate salt was used in a concentration of 60% w/v (saturation ratio) to precipitate protein in culture supernatant. The extracted crud exotoxin gave a specific activity (8.1) unit/mg , this activity was higher than the activity of a crude extract solution (6) unit/mg.

3-5-4-2- membranous infiltration (Dialysis)

Specific activity would to be higher than previous step reached (32.2) unit/mg, and also exotoxin resultant(recovery) in this step reached (46.50%) in comparison with (43.33%) in the first step of purification(3-5-4-1).

3-5-4-3- Gel filtration chromatography by using (sephadex G-100)

Clotting activity for Sa1 exotoxin at this step reached (85.5) unit/ml , specific activity (2085.3658) unit/mg , higher purified resultant according to degrees of purification reached 347.56 and exotoxin resultant(recovery) (3.975%).

Step of purification	Volume (ml)	Clotting activity (unit/ml)	Protein concentration mg/ml	Specific activity unit/mg	Total activity (unit)	Degree of purification	Exotoxin resultant (recovery) (%)
Crud exotoxin solution	500	30	5	6	15000	1	100
Precipitation by $(NH_4)_2SO_4$	200	32.5	4	8.1	6500	1.35	43.33
Dialysis	200	80.5	2.5	32.2	16100	5.366	46.50
Gel infiltration G-100	15	85.5	0.041	2085.3658	1282.5	347.56	3.975

Table(8) : Results of purification steps of Sa1 exotoxin

3-5-5- Characterization of Sa1 exotoxin

3-5-5-1- Estimation of lytic activity of Sa1 exotoxin

Sa1 exotoxin or may could named as (**staphylogen / or staphylogenic protein** according to (Werfel & Kapp , 2002)) has been showed a high lytic activity against substrate (haemoglobin). The concentration of released L-Tyrosin (0.435) mg/ml according to standard curve of Tyrosin. **Fig.(20)** .

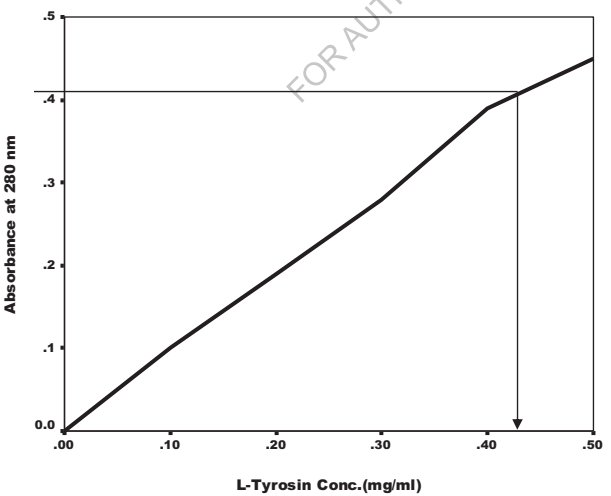


Figure (20) : Standard curve of L-Tyrosin

3-5-5-2- Some Kinetic properties of Sa1 exotoxin

Table(9) illustrated the effects various degrees of temperature and pH on Sa1 exotoxin activity. It has been found that exotoxin activity decreased in acidic and alkaline media and in high temperature ($P<0.01$) .

The optimum environmental conditions to exotoxin activity were at pH =7 and 37ºC

Table (9) : Effects of PH and temperature Sa1 exotoxin activity ($P<0.01$)

Exotoxin activity according inhibition zones (mm)			
Temperature (ºC)	PH		
	4	7	9
30	3	21	10
37	5	24	12
45	0	18	3
60	0	3	0

3-5-5-3- Cytotoxicity of Sa1 exotoxin

The minimum sa1 exotoxin concentration that showed cytotoxicity against human RBCs was 35µg/ml , so then concentration 25µg/ml –(that showed non toxic)- was used in the future study. **Table (10)** .

Table(10) : Cytotoxicity of Sa1 exotoxin against human RBCs .
T : Toxic , NT : Not toxic

Conc. µg/ml	Cytotoxicity against R.B.Cs
0.05	NT
0.5	NT
20	NT
25	NT
35	T
50	T
75	T

3-5-5-4- Antibacterial activity of Sa1 exotoxin

MICs of Sa1 exotoxin against standard bacterial strains ranged from (0.35-3.5) µg/ml , while MBCs ranged from (1-5) µg/ml. **Table(11)** . The statistical differences between the limits of MICs and MBCs were significant ($P<0.05$) .

Table(11) : Determination of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of pure Sa1 exotoxin against standard bacterial strains ($P<0.05$) .

Technique mode	Exotoxin concentration (µg/ml)			
	Staph.aureus NCTC 657	*E.coli* NCTC 5933	*B.subtilis* PCI 219	*K.pneumoniae* ATCC 10031
MIC	0.35	1.5	3.5	0.85
MBC	1.0	2.5	5.0	1.5

3-5-5-5- Evaluation of purity and molecular weight of Sa1 exotoxin by gel electrophoresis technique

Eight purified bands were obtained from *Staph aureus*1 all antigen by using polyacrylamide gel electrophoresis PAGE(7.5%). **picture (3)**, and according to comparison with a standard proteins from calibration curve **Table (12)** and **Fig.(21)**, the antigens of *Staph.aureus* 1 have a following molecular weight for each purified bands :
(549.540, 305.492, 234.422, 121.618, 58,884, 31.622, 19.186, and 13.567) kilo Dalton (kd).

While the solution of *Staph.aureus* 1 exotoxin showed a very high purified single band protein with a molecular weight of (43.315) kd . **picture (3) and Fig.(21).**

Table (12) : The relative mobility (Rm) and molecular weight of the standard proteins and various bands of *Staph. aureus* 1 (All antigens) and Sa1 exotoxin by using conventional polyacrylamide gel electrophoresis (PAGE) (7.5%)

Protein	Rm	Molecular weight (Dalton)
Thyroglobulin	0.092	669,000
Ferritin	0.184	440,000
Catalase	0.304	232,000
Aldolase	0.4	140,000
Albumin	0.569	67,000
Chemotrypsinogen	0.676	25,000
Ribonuclease	0.705	13,700
Staph.aureus 1 (All antigens): Band1	0.139	549,540.8739
Band2	0.232	305,492.1113
Band3	0.290	234,442.8815
Band4	0.406	121,618.6
Band5	0.534	58,884.3655
Band6	0.651	31,622.7766
Band7	0.744	19,186.6874
Band8	0.837	13,567.5053
Staph.aureus 1 exotoxin	0.576	47,315.1259

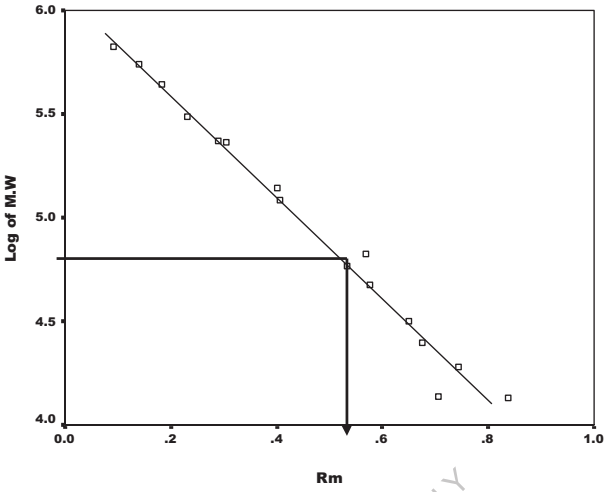


Fig- 21- : Calibration curve for estimation of molecular weight of extracted staph. aureus antigens and – in especially- exotoxin. According to comparison with standard proteins by using a conventional polyacrylamide gel electrophoresis PAGE (7.5%).

3-6- *In vivo* Study

pictures (4-11) showed all skin lesions that have been induced in mice by various techniques of infection with different infectious agents (Sa1 exotoxin, Sa1 all antigens and viable cells of *Staph.aureus1* (two doses : 25 and 50 cell /0.1ml)) .

The most common induced lesion is characterized by being erythmatous, scaly superficial and deep skin lesions in various stags of severity and chronicity. These features are clinically similar to that of atopic dermatitis and eczema in general.

3-7- Histopathological study

To confirm the evidence that the skin lesions induced experimentally in mice skin have a features typically like that of atopic dermatitis lesion, skin biopsies were performed and examined histopathologically to revealed skin changes characteristic of eczema or atopic dermatitis.

All histopathological changes were shown in **pictures (12-35)**, and we can summarize the essential changes as follows:

Pic.(13) : Skin lesion of AD of human : showed spongiosis, exocytosis with hyperkeratosis, perivascular inflammatory infiltrate consisting mainly of lymphocytes .

Mice experimentally infected gave following skin lesions :

Pic. (14) : degeneration and inflammation of epidermal layers .

Pic. (15) : hyperkeratosis, spongiosis with infiltration of inflammatory cells reaction with increase of blood vessels & nerves in dermis .

Pic. (16) : Infiltration and diapedes movement of inflammatory cells blood vessels with edema in the dermis and hypodermis.

Pic. (17) : Subcutaneous adipose tissues necrosis with inflammation in the dermis.

Pic. (18) : Surface ulceration , severe inflammation in epidermal and dermal layers.

Pic. (19) : Inflammatory cells in subcutaneous tissues between muscle fibers.

Pic. (20) : Infiltration of inflammatory cells in skin layers and hair follicles .

Pic. (23) : Sub epidermal cleft, degeneration and inflammation in skin layer, with high presence of blood vessels and nerves.

Pic. (26) : spongiosis, hyperkeratosis and mild inflammatory cells reaction in dermal layers.

Pic. (27) : Acute inflammation in subcutaneous layer of skin .

Pic. (28) : Moderate inflammation, edema, abscess formation and necrosis in the dermis layer and hypodermis.

Pic. (29) : hyperplasia (acanthosis) in epidermis .

Pic. (30) : Moderate inflammation with edema between collagen fibers in lower dermis.

Pic. (31) : Mild acute dermatitis .

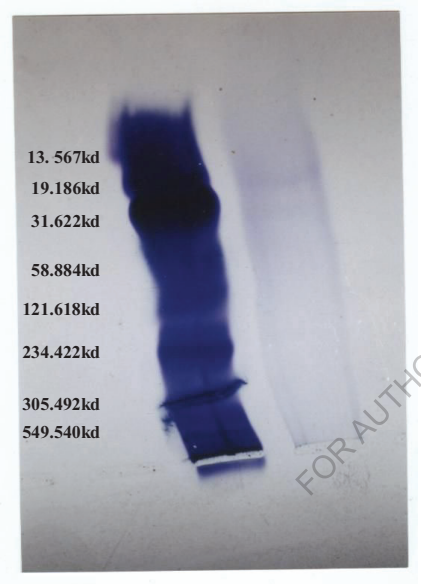
Pic. (32) : Acanthosis , spongiosis and inflammation in the dermis.

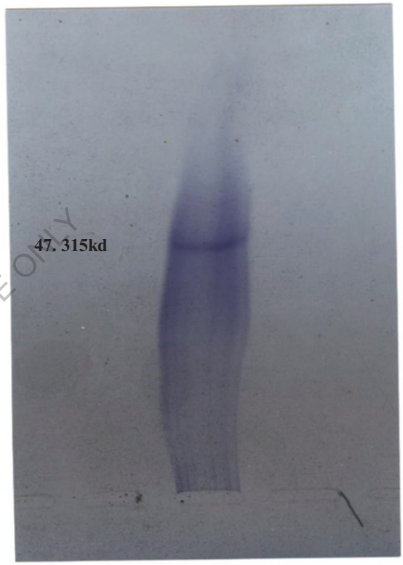
Pic. (33) : Intradermal bullous formation in the dermis.

Pic. (34) : Neutrophils in deep dermis layer.

Pic. (35) : **Normal mice skin, control** .

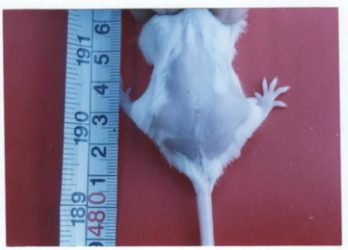
PICTURE (3) : BANDS FROM GEL ELECTROPHORESIS .
LEFT : *STAPH AUREUS* ALL BODY ANTIGENES .
RIGTH : SINGLE PURE BAND OF *STAPH AUREUS* EXOTOXINE (SUPERANTIGENE)





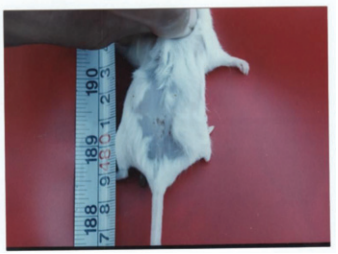
PICTURE (4): CONTROL .

PICTURE (5): SKIN LESION IN 24HRS AFTER INTRADERMAL INJECTION OF EXOTOXIN .

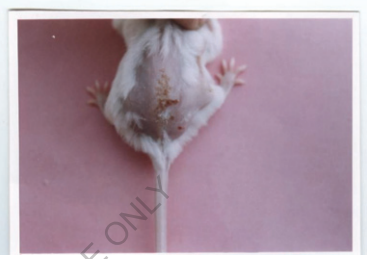


PICTURE (6) : SKIN LESION IN 48HRS AFTER PRICK TEST OF EXOTOXIN (SUPERANTIGENS) .

PICTURE (7) : SKIN LESION AS A TYPICALLY PHENOMENA OF AD (SCALY & ERYTHMATOUS) IN 96 HRS AFTER SPOT TEST OF EXOTOXIN .



PICTURE (8) : SKIN LESION IN 48HRSAFTER SPOT TEST OF 50 CELL / 0.1 ml OF *STAPH . AUREUS*



PICTURE (9) : SKIN LESION IN 10 DAYS AFTER SPOT TEST OF 25 CELL / 0.1 ml OF *STAPH . AUREUS*

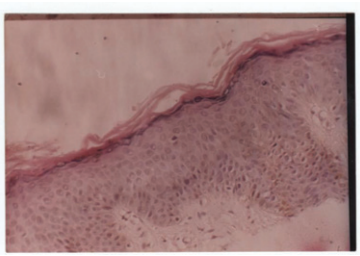
PICTURE (10) : SKIN LESION AS A TYPICALLY PHENOMENA OF AD (SCALY & ERYTHMATOUS)

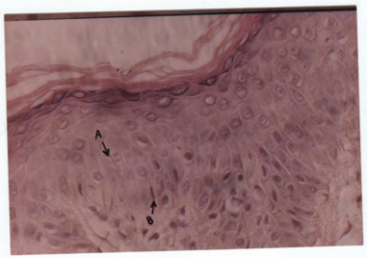
PICTURE (11) : SKIN LESION IN 15 DAYS AFTER SPOT TEST OF *STAPH . AUREUS* ANTIGENS . IN 120 HRS AFTER INTRADERMAL INJECTION OF *STAPH . AUREUS* ANTIGENS .

PICTURE (12) : HUMAN SKIN , CONTROL . 66x

PICTURE (13) : SKIN LESION OF HUMAN AD . A : SPONGIOSIS B : EXOCYTOSIS .

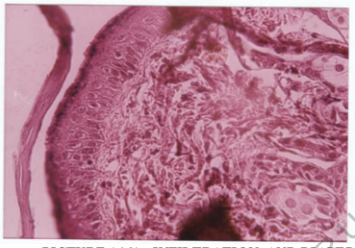
WITH HYPERKERATOSIS . 132x

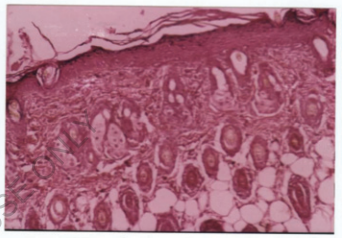




PICTURE (14) : DEGENERATION AND INFLAMMATION OF EPIDERMAL LAYERS . MICE SKIN LESION . 65x

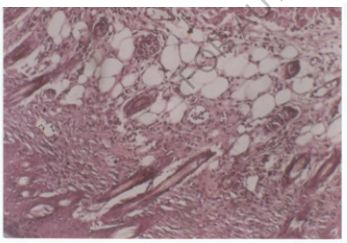
PICTURE (15) : HYPERKERATOSIS , SPONGIOSIS WITH INFILTRATION OF INFLAMMATORY CELLS REACTION WITH INCREASE OF BLOOD VESSELS & NERVE IN DERMIS . 26x

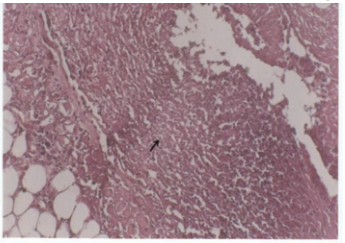




PICTURE (16) : INFILTRATION AND DIAPEDES MOVEMENTOF INFLAMMATORY CELLS FROM BLOOD VESSELS WITH EDEMA IN THE DERMIS & HYPODERMIS . 26x

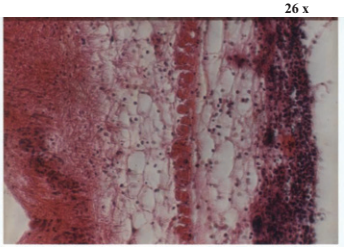
PICTURE (17) : SUB CUTANEOUS ADIPOSE TISSUES NECROSIS WITH INFLAMMATION IN THE DERMIS . 26x

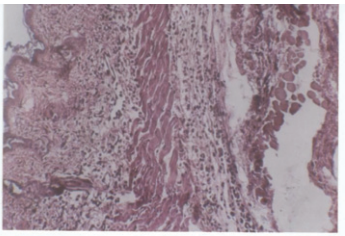




PICTURE (18) : SURFACE ULCERATION , SEVERE INFLAMMATION IN EPIDERMAL & DERMAL LAYERS . 26 x

PICTURE (19) : INFLAMMATORY CELLS IN SUBCTANEOUS TISSUES BETWEEN MUSCLE FIBERS . 26 x

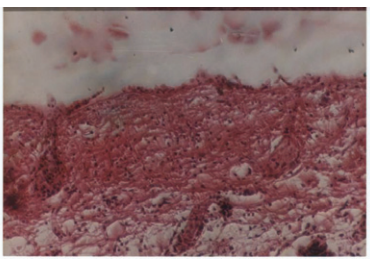




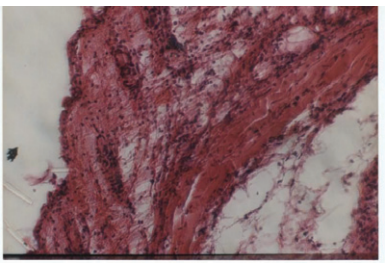
PICTURE (20) : SURFACE ULCERATION , INFILTRATION OF INFLAMMATORY CELLS IN SKIN LAYERS & HAIR

PICTURE (21) : SURFACE ULCERATION , SEVERE INFLAMMATION WITH EDEMA IN SKIN LAYERS

FOLLICLES . 26 x

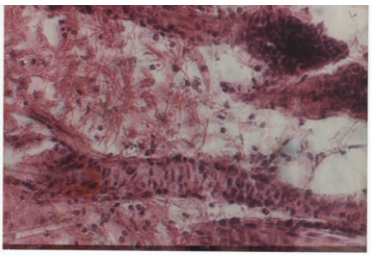


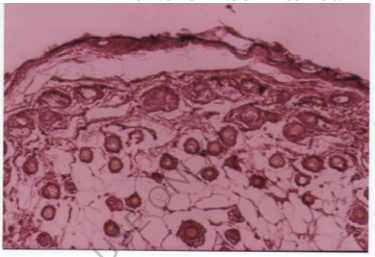
26 x



PICTURE (22) : INFILTRATION OF INFLAMMATORY CELLS IN HAIR FOLLICLES . 132 x

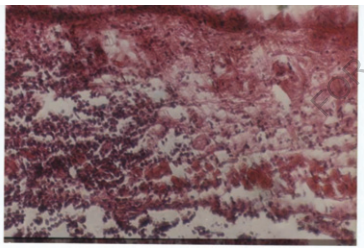
PICTURE (23) : SUB EPIDERMAL CLEFT , DEGENERATION & INFLAMMATION IN SKIN LAYERS WITH HIGH PRESENCE OF BLOOD VESSELS & NERVES . 26 x

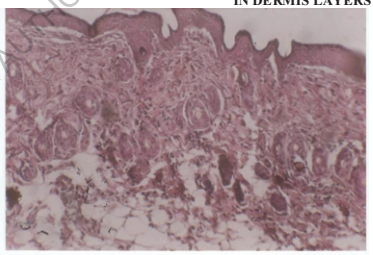




PICTURE (24) : SURFACE ULCERATION WITH SEVERE FLAMMATION & EDEMA . 66x

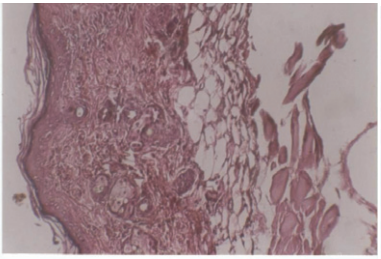
PICTURE (25) : EDEMA , INFILTRATION OF INFLAMMATORY CELLS WITH AQUEOUS INFILTRATION BETWEEN CELLS IN DERMIS LAYERS . 26 x

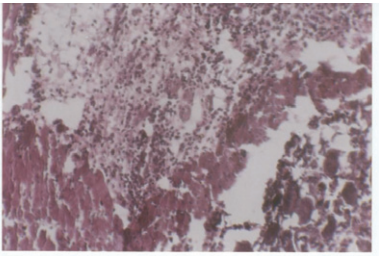




PICTURE (26) : SPONGIOSIS , HYPERKERATOSIS AND MILD INFLAMMATORY CELLS REACTION IN DERMAL

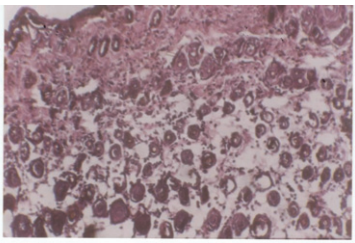
PICTURE (27) : ACUTE INFLAMMATION IN SUBCUTANEOUS LAYER OF SKIN . 65 x LAYERS . 26 x



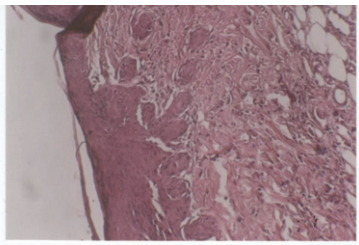


PICTURE (28) : MODERATE INFLAMMATION , EDEMA , PICTURE (29) : MILD INFLAMMATORY CELLS

ABCESS FORMATION AND NECROSIS IN THE DERMIS LAYER & HYPODERMIS . 26 x

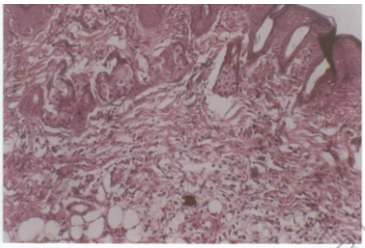


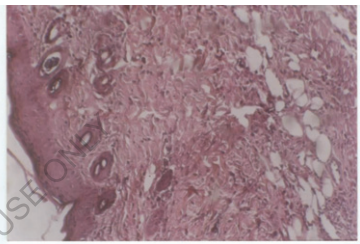
REACTION WITH HYPERPLASIA (ACANTHOSIS) IN EPIDERMIS . 26 x



PICTURE (30) : MODERATE INFLAMMATORY CELLS REACTION WITH EDEMA BETWEEN COLLAGEN FIBERS

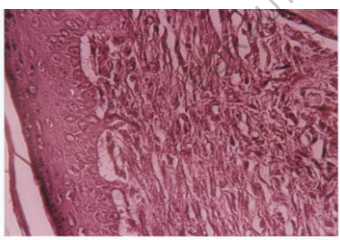
PICTURE (31) : MILD ACUTE DERMATITIS WITH INFLAMMATION AND EDEMA . 26 x IN LOWER DERMIS . 26 x

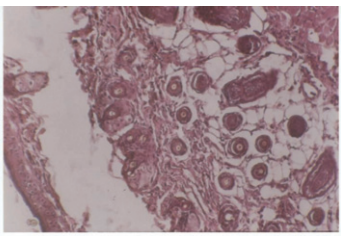




PICTURE (32) : ACANTHOSIS , SPONGIOSIS AND INFLAMMATION IN THE DERMIS . 65 x

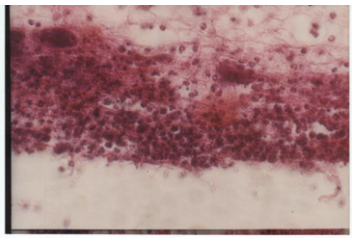
PICTURE (33) : MILD INFLAMMATION REACH TO SUBCUTANEOUS AREA WITH EDEMA AND INTRADERMAL BULLOUS FORMATION IN THE DERMIS . 26 x

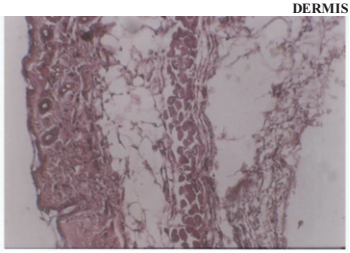




PICTURE (34) : INFILTRATION OF INFLAMMATORY CELLS -IN ESPECIALLY – NEUTROPHILES IN DEEP

PICTURE (35) : MICE SKIN , CONTROL 26 x DERMIS LAYER . 132 x





Chapter four

Discussion

4-1- Epidemiological and clinical studies :

In this study , from a total of (484) AD patients we found that the prevalence of AD in male and female were (43.6% and 56.4%) respectively with male: female ratio (1:1.29). The infantile and childhood age groups recorded a higher prevalence (34.7% and 27.5%) respectively than the adult age groups (37.9%) .

In other view , male was highly affected with AD in stages (1,4,5) while female was highest in stages (2&3) in percentages (55.36 , 43.28 , 32.48 , 61.65 and 60.35%) respectively .

Many recent studies interested in skin diseases couldn't diagnose atopic dermatitis from them survies in Iraqi districts such as (AL-Ibadi , 1993 , Abdul majeed , 2002 , and Al-mahfouz , 2003) , while Affat (1998) mentioned to atopic dramatis depending on the presence of erythema . So , no previous study in Iraq or in nearest countries was found to deal alone with atopic dermatitis as a clinical skin disease and /or interested in the diagnosis according to the major and minor clinical criteria, – in addition – no studies in Iraq and other arabic countries, detailed atopic dermatitis as a bacterial complications , determination of bacterial agents , and determination of the main causes of atopic dermatitis .

So , this study is the first one , at least in Iraq , that dealt with all questionable information about atopic dermatitis .

To support this evidence , we make use of some studies a bout skin infection in some arabic countries such as (Qadri , *etal* ., 1990 ; Bahamdan , *etal* , 1996 ; Parthasaradhi & ALGufai , 1998 and Shakkoury & Wandy , 1999) .

Many studies supported the results of our study ; a slight female preponderance has been demonstrated in some studies (Dotterud , *etal*. , 1999; Schäfer , *etal*. , 1996 ; Herd , *etal*. , 1996 ; Marks , *etal*., 1999 and Laughter , *etal*. ,2000) . A small female preponderance was also noted in the (International Study of Asthma and Allergies in Childhood) (ISAAC) study with an overall female : male ratio of 1.3:1 being higher in countries with the highest prevalence of atopic dermatitis symptoms . (Charman & Williams, 2002) .

Our results interested in age related - atopic dermatitis confirmed by study of Sugiura , *etal*. (1998) that found the a topic dermatitis is predominantly a disease of infancy and childhood , and prevalence estimation showed a continuous reduction with increasing age . A recent Norwegian study based on a review of medical records revealed that the prevalence of atopic dermatitis of in patients under the age of 20 years was 13% compared to (2%) for those over the age of 20 years (Falk ; 1993 and Wuethrich, *etal*., 1990) .

In the United Kingdom (2%) of adults aged 16-40 years and less than (0.2%) of adults over the age of 40 years are affected with AD . (Herd , *etal*., 1996) .

The distribution of atopic dermatitis changes as the infant grows older concomitant with crawling and involvement of all surface of the limbs becomes evident . (Leung , 1996) . The disease than runs a chronic fluctuating courses ; exacerbation has been associated with such factors as teething , respiratory infections and emotional upsets , the remainder continue their pattern of puberty . (Hanifin , 1990,1991 and Flohr , *etal*., 2005).

The itchiness of atopics is often noted as early as the newborn period , by their increased crying and restlessness , in the nursery , and the age of onset of AD has been well documented by many researchers . (Leung , 1999 ; Weilan, *etal*., 1999 and Flohr , *etal*., 2005) .

There is a correlation between early age of atopic dermatitis and its severity , the earlier age pottends a more severe course (Svensson ,1989). Approximately (60%)

of patients are noted to have AD before their first birthday. In another (30%) of AD is before the age of five (Zeiger , *etal*., 1992).

This study revealed a high correlation between the family and personal histories with atopic dermatitis . The early studies to determine whether or not atopy has a genetic components involved ascertaining the prevalence of allergic diseases in first – degree relatives of atopic individuals . (Ono , 2000) . A prospective study of several hundred newborns over a 5 years period gave a more convincing evidence that atopic dermatitis and other forms of atopy were passed from parent to children , fifty one percent of children with a family history of atopy compared to (19%) of children with no family history of atopic developed symptoms of allergic disease within the first 5 years of life . (Luoma , *etal*., 1983 and Laitinen , *etal*., 2000) . Dold , *etal*. (1992) found that children of parents with atopic dermatitis are more likely to develop atopic dermatitis than children of asthmatic parents or those with allergic rhinitis . This could be interpreted as the genetic component manifesting itself in a tissue – specific manner , suggesting that genes specific for atopic dermatitis might exist (Schultz , 1993 ; Forrest , *etal*., 1999 . and Flohr , *etal*., 2005) .

In our results , the types of skin (dry, seborrheic and natural) found to be associated ,in various degrees , with atopic dermatitis, but , the dry type of skin was a predominant in male & female and its more associated with AD. This may be due to lower contents of ceramide and sphingomyeline in the skin of AD patients (Barth , *etal*., 1989 ; Aalto , 1995 ; Bouwstra , *etal*., 1998 and DiNardo , *etal*.,1998) .

The epidermal permeability barrier resides in the stratum corneum (SC) , a heterogeneous , two – compartment tissue . Whereas the cells (corneocytes) of the SC are lipid – depleted , they are embedded in a continuous , lipid – enriched extra cellular matrix organized into characteristic , multilamellar membrane unit structures , which mediate barrier function (Elias & Menon , 1991 and Schurer , *etal*., 1991) . The formation of the permeability barrier is the goal of epidermal proliferation and differentiation, processes that begin in the basal layer . The quantitatively most important cell type of the epidermis , the keratinocyte, derives from stem cells , and en route to the SC it synthesize specific basal (K_5 and K_{14}) and supra basal (K_1 and

K_{10}) keratins , as well as cornified envelope (CE) – associated proteins . (Doering, *etal*., 1999 and Horrobin , 2000) .

An alternative explanation for the decrease in ceramide content in AD patients was provided by Ohnishi , *etal*., (1999) who found that more ceramidase was secreted from bacterial flora of both lesional and non lesional skin of AD than normal subjects , so , the skin of AD becomes dry . These results of Ohnishi and co – workers strongly confirmed our results which found a high correlation and distribution of dry skin in AD patients ($P< 0.001$) .

Many other studies (Tupker , *etal*., 1990 ; Jin , *etal*., 1994 ; Gfesser , *etal*., 1997 ; Hara , *etal*., 2000 and Uchida , *etal*., 2000) suggested that the skin of patients with AD is colonized by ceramidase – secreting bacteria , and that these microorganisms contribute to the ceramids – deficiency in AD . Thus , comprehensive studies on lipid metabolism in AD suggest that a decrease in ceramides is the cause of the impaired permeability barriers in AD . (Yamamoto , *etal*., 1991 and Ohnishi , *etal*., 1999) .

In addition , the study pointed clearly to the role of bacteria as a cause of skin dryness in AD patients .

4-2- Haematological study :

This results showed that values of Hb , RBCs count and basophiles were under the normal or control means ($P < 0.001$) while values of ESR , WBCs count , lymphocytes count , eosinophils , and platelets count were higher than normal means ($P<0.001$) . On the other hand , the percentages of neutrophils , lymphocytes , and monocytes were within normal means ($P\geq0.05$) .

The relationship between blood components and atopic dermatitis had not been evaluated by previous studies . But there are a few reports bout relations of some blood components with allergic diseases or reactions and crossly with atopic dermatitis . Firstly Mast cells and basophiles are generally recognized as the principal cell types to initiate IgE – dependent , type 1 immediate hypersensitivity reactions . The role of mast cells and basophiles in allergic disorders is well established , but their significance in atopic dermatitis is still remains unclear . (Kambe , *etal*., 2002) .

Several studies suggest a relationship between atopic dermatitis and basophiles , lesions of atopic dermatitis where lichenified , the number of basophiles may be increased in patients with AD, although no significant correlation between clinical severity scores and basophile numbers was found (Irani , *etal.,* 1989 ; Lantz , *etal.,* ; 1998 and Wedemeyer , *etal.,* 2000). Moreover , patients suffering from atopic dermatitis have markedly elevated levels of serum IgE . Mast cells and basophiles are well-known target cells for this molecule , because they express abundant amount of Fcε RI on their surface (Caughey , 1995) . In addition , certain genes associated with atopy are also expressed by mast cells and basophiles (Stanford , *etal.,* 1993 and Shirakawa , *etal.,* 1994) . Mast cells and basophiles contain numerous potent different effects in inflammation at sites of their activation , mediators secreted by activated mast cells and basophiles can be divided into those stored in secretary granules prior to cells activation and those that are newly generated after an activation signal . The former include : histamine proteoglycans , proteases , and certain cytokines ; while the latter include metabolites of

arachidonic acid , cytokines , and chemokines (Hannuksela , *etal.,* 1993 .and Macfarlane , *etal.,* 2000) .

All these mediators play a role in the pathogenesis of skin lesions as atopic dermatitis and others. These data confirming our results , showing the correlation of AD with elevated basophils count .

The most important role of blood components with AD was that of eosinophils which was elevated in (76.7%) of AD patients in our study . These results were compatible with all preview studies that suggested prominently strongly association with allergic reactions . Also , investigations have shown that eosinophils elaborate potent toxins and mediators of inflammation . Eosinophils also play an important role in the switch from TH_2 to TH_1 lymphocytes in the AD lesions via production of IL-12 (Wuthrich & Grendelmeier , 2003) .

The evidences for eosinophils involvement in atopic dermatitis are :

1. Eosinophils and eosinophil granule proteins possess phlogistic activities and cytotoxic effects that are associated with allergic inflammation . (Leiferman , 1994) .

2. Eosinophils granule proteins are extensively deposited in lesional skin with evidence of cytolytic eosinophil degeneration . (Moqbel , 1994) .

3. Eosinophil granule proteins are increased in peripheral blood and correlate with disease activity . (Leung , 1992) .

4. Peripheral blood eosinophils are increased in server disease which decrease with therapeutic improvement ; "activated" hypodense , eosinophils with prolonged survival , i.e. , delayed programmed cell death , correlate best with disease activity . (Hansel , *etal.,* 1993 , and Leung ,1999) .

5. Th_2 immunological reactivity present in atopic dermatitis is associated with IL-5 expression ; IL-5 has specific activities on eosinophils inducing eosinophilopoiesis , activation , and chemotaxis . (Rothenberg , *etal.,* 1989 and Kita , *etal.,* 1992) .

6. Adhesion molecule expression needed for eosinophil transendothelial migration is present in a topic dermatitis . (Kaplan , *etal.,* 1995) .

7. Eosinophil chemotaxins are expressed in atopic dermatitis (Schröder, *etal.,* 1996) .

8. Eosinophil infiltration and extracellular eosinophil granule protein deposition occur in patch test allergen models of atopic dermatitis . (Abu-Ghazaleh , *etal.,* 1992 , and Langeveld , *etal.,* 2000) .

4-3- Immunological study :

The concept that AD has an immunologic basis is supported by the observation that patients with primary T-cell immunodeficiency disorders are frequently associated with elevated serum IgE levels and eczematoid skin lesions indistinguishable from AD (Leung , 1992 , and Leung & Hamid , 1996) . Also , non atopic recipients receiving bone marrow transplants from atopic donors develop positive immediate skin tests and atopic symptoms following successful engraftment . (Agosti , *etal.,* 1988). These data indicate that AD is not due to a constitutive skin defect but is instead mediated by a bone marrow-derived cells .

This study showed highly significant elevation of various types of studied clusters of differentiation (CD) values . These results were compatible with results of other studies such as (Akdis , *etal.,* 1999 , 2000 ; Wakugawa , *etal.,* 2001 ; Seneviratne , *etal.,* 2002 ; Rossje , *etal.,* 2002 ; Ohshima , *etal.,* 2002 ; Leung & Bieber , 2003 and Lima , *etal.,* 2003) that evidenced a marked correlation between various CD_s and atopic dermatitis where CDs values were elevated in AD patients .

While , many other studies detailed other CD_s in relation with AD, such as CD_{26} , CD_{23} , CD_{24} , CD_{26} , CD_{28} ,CD_{30} , CD_{45} , CD_{83} , CD_{137} and CD_{153} (Ellis , *etal.,* 1993 ; Dummer , *etal.,* 1997 ; Gilfillan , *etal.,* 1998 ; Mallet , *etal.,* 1999 ; Langstein , *etal.,* 1999 ; Katoh , *etal.,* 2000 ; Qymar , *etal.,* 2000 and Bengtsson , 2001) .

Cluster designation of monoclonal antibodies (Clusters of differentiation (CD)) designated from 1st to 8th workshop on international human leukocyte differentiation antigens with total number of (247) CD_s . Leukocytes express distinct assortments of molecules on their cell surfaces , many of which reflect either different stages of their lineage-specific differentiation or different states of activation or inactivation . Leukocyte cell surface molecules are routinely detected with anti-Leukocyte monoclonal antibodies (mAbs) . Using different combination of mAbs , it is possible to chart the cell surface immunophenotypes of different Leukocyte subpopulations , including the functionally distinct mature Lymphocyte subpopulations of B-cells , helper T-cells (T_H) , cytotoxic T-cells (T_c) , and natural killer (NK) cells . (Sears , 1997) .

The essential functions of studied CD_s are : **CD_3** ; Associated with T-cell antigen receptor . Required for cell surface expression of and signal transduction by TCR . Cytoplasmic domains contain ITAM motifs and bind cytoplasmic tyrosine Kinase .(Kisielow & Boehmer , 1995)

CD_4 : Coreceptor for MHC class II molecules . Binds Lek on cytoplasmic face of membrane . Receptor for HIV-1 and HIV-2 (Tonneau , 1999) .

CD_8 : Coreceptor for MHC class I molecules . Binds Lek on cytoplasmic face of membrane. (ZLI,2004)

And **CD_{19}** : forms complex with CD_{21} (CR_2) and CD_{81} (TAPA-1) , coreceptor for B cells-cytoplasmic domin binds cytoplasmic tyrosine kinases and PI-3 kinase. (Sato & Tedder, 1999).

The explanation about elevation of CD values in AD patients is correlated with their function and interrupted with allergic disorders according to that most of patients with AD have elevated levels of serum IgE and elevated numbers of circulating eosinophils, reflecting an increased expression of the cytokines (Leung,1995). Indeed, a number of studies have demonstrated increased frequency

of allergen-specific T cells producing increased IL-4, IL-5, and IL-13, but Little IFN-α in the peripheral blood of patients with AD. (Kimura,*etal.,*1998;Dummer,*etal.,*1998;Nikoloff,1999and Cavagni, *etal.,* 2000).

Numbers studies pointed to the role of activated CD_4^+ and CD_8^+ T cells in atopic dermatitis and other allergic inflammatory diseases. (Akdis & Akdis 2000). Systemic activation of Tcells in AD is supported by observation that these patients possess increased numbers of activated cutaneous lymphocyte associated antigen (CLA) –bearing Tcells in the circulation and increased levels of serum L-Selectin, a marker for leukocyte activation correlating with AD disease severity (Santamaria, *etal.,* 1995, Akdis, *etal.,* 1997 and Shimada, *etal.,* 1999) . Dermal cellular infiltrate in AD mainly consists of CD_4^+ and CD_8^+ Tcells with a CD_4/CD_8 ratio similar to peripheral blood levels (Akdis, *etal.,* 1999,2002). In recent studies, CD_8^+ CLA^+ Tcells were demonstrated to be as potent as $CD_4^+CLA^+$ Tcells in induction of IgE and inhibition of eosinophil survival (Akdis,etal.,1999).

Kehry and Yamashita (1989) have shown that IgE bound to CD_{23} and others CD on mouse B cells may be used to focus antigen to T cells. This mechanism operates selectively at extremely low doses of allergen to CD_4^+ and CD_8^+ T lymphocytes is greatly enhanced by IgE-facilitated antigen presentation (Santamaria,1993, and Neerven, *etal.,* 1999).

Our results revealed that all means of concentrations of Immunoglobulin (Ig) (IgA, IgG and IgM) and complement components (C_3 and C_4) were increased more than those for control group in high significant percentages.

This findings were compatible with the results of other studies such as (Somos & Schneider, 1993; Morsy, *etal.,* 1994and Hill, *etal.,* 1995) that found elevation of Immunoglobulin concentrations of AD patients and some experimentally AD animals , also Senol, *etal.* (1997) found a similar elevation of Ig_s and complement components in population aged 1-63 years, and the IgG, IgM, IgA, C_3 and C_4 reached (1992.49, 188.91, 264.36, 157.91 and 29.12) (mg/dl) respectively , and concluded that immunity to atopic skin diseases involved mainly humoral, especially IgE-mediated, immune response but this syndrome is also associated with

a cell-mediated immunological response against many kinds of exogenous and endogenous factors, or a non specific reaction (Bjerke,1994, and Okada, *etal.,* 1996).

Other recent studies interested in one or two types of immunoglobulins or special modes of immunity and agreed with our results to confirm the elevation of Immunoglobulins and complement components in various regions of the world (kirkeby, *etal.,* 2000 ; Geha , 2001 ; Schiessl, *etal.,* 2001; Leung & Soter, 2001 ; Wenzel & Bieber, 2004 and Szakos, *etal.,* 2004).

The most important role to interrupt with in the immunopathology of AD was that for IgE. According to the results of the present study IgE concentration was elevated in 86.5% of AD patients . And our study grouped AD patients according to modes of allergy into three group, in which it was found that the mode of Allergy very probable (IgE >100 IU) was predominant in (88.8%) of various age groups of AD patients.

Most of previous studies found that serum IgE levels were elevated in 80-85% of patients with AD. (Quinti, *etal.,* 1986;Bieber, *etal.,* 1992; Chan & Hanifin, 1993; Leung, *etal.,* 1993;and Leung & Hamid, 1996; Matsuda, *etal.,* 1997; Kita & Gleich, 1997; Natter, *etal.,* 1998; Bunikowski, *etal.,* 1999; Cavallo, *etal.,* 2002; Edwards, *etal.,* 2002; Ledin,2004 and Perkin, *etal.,* 2004) .

In normal people , circulating IgE concentration is very low because mast cells have a very high affinity for IgE. The synthetic rate for IgE is also very low. IgE attaches to the mast cells and basophils and activate the eosinophils. IgE on mast cells has a half life of more than 10 days (Leung, *etal.,* 2004) . Mast cells play several roles in host defense . As the origin of many proinflammatory substances, mast cell degranulation , mediated inflammation & allows circulating cells and plasma proteins to increase access to interstitial spaces to combat infection when antigens (or in the case of allergies and atopic dermatitis (allergens)) cross – link IgE on mast cell surfaces , mast cell degranulation is triggered. Degranulation releases histamine and other biologic substances that can induce coughing, sneezing, vomiting, or diarrhea. These actions serve to expert pathogens from the body. (Leung, 2000 and Goldsby, *etal.,* 2003).

In the present study four environmental (food , fungal , agricultural , and aeroallergens) and eleven extracted bacterial antigens (as allergens) were tested against specific IgE. We found a very high significant allergen – specific IgE reactions with a high degrees mediated a various forms of hypersensitivity in AD patients, - inespecially- *Staph.aureus*1 exotoxin could be named as (**staphylogen or staphylogenic proteins**) that recorded the highest degree (100%) for mode of hypersensitivity(B ,A, H and over).

Our result interested in allergen – specific IgE reactions which were absolutely compatible and agreed with the results of previous studies that have been carried out about atopic dermatitis as follows :

4-3-1- Food allergens – specific IgE reactions :

Serum IgE levels are elevated in 80-90% of AD children, and positive immediate skin tests and RASTS to various dietary and environmental allergens are present in approximately 80% of children with AD (Sampson,1992). Identification and strict avoidance of relevant allergens has been of significant therapeutic benefit (Grunther & Sampson, 2002). Food hypersensitivity has been shown to cause worsening of skin symptoms in 33-40% of children with moderate to severe disease (Burks, *etal.,* 1988 and Eigenmann, *etal.,* 1998). Eigenmann (2000) reported that out of 74 Swiss children referred to a pediatric allergist or dermatologist for atopic dermatitis, 34% of them had hypersensitivity.

Many types of food allergens have been studied that were found to cause or related with atopic dermatitis in various ages such as :

Proteins (David, *etal.,* 1984 ; Spergel, *etal.,* 1998, and Schade, *etal.,* 2000) .

Fishes (Hill & Lgnch, 1982; Jones & Sampson, 1996 and Niggemann, *etal.,* 2000) .

Wheat (Sampson & McCaskill, 1985; Sampson & Scanlon, 1989; Sampson, 1997, and Majamaa, *etal.,* 1999).

Milk(David, *etal.,*1984; James & Sampson ,1992; Suomalainen,1993, Abernathy, *etal.,* 1995; Werfel, *etal.,* 1997 , and Schade,2000).

Egg (Fergusson, *etal.,* 1983; Zeiger, *etal.,* 1989; Sigurs, *etal.,* 1992; Sampson, 1997; Lever, *etal.,* 1998 and Li, *etal.,* 2001).

And others (Peanut, Soy, Vegetables, tree nut, Seafood, and others) (Burks, *etal.,* 1988; Sampson, 1992, 1997; Niggemann, *etal .,* 2000 ; Worm, *etal.,* 2000 and Grunther & Sampson, 2002) .

4-3-2- Aeroallergens :

Recent studies suggested that the quantity of specific IgE to various allergens was compared in serum assays of patients with atopic dermatitis. e.g. the great test concentration of IgE was specific for dust mite. In fact concentrations of IgE to dust mite allergens in patients with AD were in general at least 20-fold higher than those found in sera from patients with asthma (Scalabrin, *etal.,* 1999). Same of these results were found in our study that evidenced the strongly relation between various aeroallergens and the concentration of specific IgE .

Modern studies suggest a linkage between type I and cellular hypersensitivity mechanisms, which proposed a role of IgE bearing langerhans cells. They hypothesized that aerollergens could penetrate the skin and bind to IgE on langerhans cells contributing to local T- lymphocyte response. T lymphocytes could be involved in induction of the eczematous response as well as being responsible for production of IL-4. (Bruijnzeel, *etal.,* 1986., Ring, *etal.,* 1997., Platts & Woodfolk, 2000 ,and Irani, *etal.,* 2001) . In addition to its role in the production of IgE by B cells, IL-4 may be involved in induction of $F_c\varepsilon RI$. This can be expressed on monocytes, which differentiate into dendritic cells, or directly on dendritic cells, thus amplifying the response to aeroallergens (Bruijnzeel, *etal.,* 1989). Some other studies continue to argue that it is possible that elevated IgE levels in AD patients are simply an incidental finding or are a consequence of altered B cell regulation. However the strength of the association with IgE antibodies to common inhalant allergens strongly argues that they are directly relevant to the pathogenesis to atopic dermatitis. (Chapman, *etal.,* 1983; Clark & Adinoff, 1989; Wuthrich, *etal.,* 1990; Robert, 1994; Tan, *etal.,* 1996; Liu, *etal.,* 2000; Platt & Woodfolk, 2000 and Erwin & Platt, 2002) .

4-3-3- Fungal Allergens :

Fungi constituting microflora of human body like *Candida albicans , Malassezia, Aspergillus, Pencillium* and other air borne molds, contain various antigenic components such as polysaccharides and proteins. They induce Th-1 and/or Th-2 dependent immune responses if they penetrate the skin. (Tanaka , *etal.,* 1994; Ring, *etal.,* 1997; Morita, *etal.,* 1999, and Tengvall, *etal.,* 2000).

Other studies suggested that, after continual exposure to fungal allergens for prolonged period, AD patients seem to show a shift from a Th1 and Th2 lymphocytes dominance of the immune response even to an allergen that will normally stimulate Th1 lymphocytes to induce contact sensitivity in most individuals. Although fungal allergy in AD patients may be not the primary cause for their skin disease, it can be an aggravating factor. (Lindgren, *etal.,* 1995 and Hiruma , *etal.,* 1999) .

4-3-4-Bacterial antigens and superantigen (Staphylogen or exotoxin):

Most previous studies interested in *Staph aureus* and AD , have not published data concerning extraction of antigens from bacterial flora associated with AD and its introduced as allergens against specific IgE, however, our view about this disappoint suggest that all of microbial flora on the AD skin lesion may cause or contribute with the inflammation process of atopic dermatitis according to high percentages of isolation in both healthy area and eczematous lesions in patient with AD in comparison with a control group. (Aly , 1980; Feingold,1986; Williams, *etal.,* 1990; Strange, *etal.,* 1996, Nomura, *etal.,* 1999, Strickland, *etal.,* 1999 and Werfel ,2005) .

Differences in sebum and sweat secretion and increased bacterial adhesion to epithelial cells in atopic skin diseases may predispose to enhanced amount of *Staph.aureus* on the skin. (Senol, *etal.,* 1996) . *Staph.aureus* secrets exotoxins as superantigens, which stimulate a large proportion of Tcells. On the other hand, Staphylococcal antigens may induce allergic reactions (ie , the release of inflammatory mediators such as leukotrienes and histamine) (Leung, *etal.,* 1993) . in addition , protein A, a component of the cell wall of *Staph. aureus* is a potent B

cell mitogen (Williams & Mackie,1993). This understanding provides a rationale for attempting to reduce the Staphylococcal skin colonization of patients with atopic dermatitis and correlates with the clinical observation in a number of situations of marked improvement in atopic dermatitis following antibiotic treatment (Number, *etal.,* 1993; Ogawa, *etal.,* 1994 ; Misko, *etal.,* 1995 and Kemp & Campbell, 1996).

4-4- Bacteriological Study

The present study reveals a positive cultures from the total AD patients (eczematous lesions and healthy skin) in 94.4% and 86.36% respectively . Twenty various bacterial types and a total of (959 and 744) isolates were identified from each above mentioned sites respectively. *Staph. aureus* was a predominant bacterial agent isolated in (60.48% and 17.48%) from the same above mentioned sites respectively followed by other bacterial types in various percentages of isolation .

Also , the modes of isolation were studied ,we found that , two bacterial agents was predominant in both above mentioned sites while (six and more) mode of isolation recorded (33.52% and 6.88%) from each of the studied sites respectively that indicate a high prevalence of bacterial types in eczematous lesion in comparison with healthy skin .

To confirm these evidence , a total number of bacterial types was determined for AD patients and found to be ranged from 0.02-92.0x 10^5 cell/cm in eczematous lesions and 0.11-23.0x10^3 cell/cm in nearly healthy skin. ($P<0.001$) .

The results of previous studies were compatible with ours. Discussions on the importance of microbial factors on the pathogenesis of eczema and therapeutical implications for the treatment of atopic dermatitis began more than 100 years ago. (Abeck & Mempel, 1998). Since then, our knowledge concerning the complex interaction between microbes and skin inflammation has improved dramatically and today the Gram positive bacterium, *Staph.aureus* is recognized as an important

triggering factor for the maintenance of skin inflammation and acute exacerbations of the genetically determined skin disease as atopic dermatitis (Dahl,1983; Leyden, *etal.,* 1993 and Strange, *etal.,* 1996)

Recent studies that agreed with our study included Brook, *etal*. (1996) who found that (36% ,20% ,44%) of aerobic ,anaerobic ,and mixture of both respectively isolated from AD patients, these bacteria were : *Staph .aureus ,* Group A-streptococci, *E.coli, Peptostreptococcus , Prevotella , Porphyromonas* spp .and *Fusobactrium* .Morishita, *etal.* (1999) noticed a heavy colonization of AD with *Staph aureus* ,this phenomenon suggests that *Staph. aureus* in AD lesions influences the disease processes . Breuer, *etal.* (2000) demonstrated that , the colonization density of eczematous lesion can reach 10^7 colony forming units/cm^2 . Breuer, etal. (2002) found that the skin of 100% of patients with atopic dermatitis is colonized with *Staph. aureus*, up to 65% of all *Staph.aureus* strains isolated from lesional skin have been shown to produce exotoxins with superantigenic properties. Heaton, *etal.* (2003) illustrated that the incidence of *Staph. aureus* colonization on the skin of patients with AD is approximately 90%.

Our results showed an increase in the incidence of *Staph.aureus* colonization in eczematous lesions compared with healthy skin in (173 (60.48%) , 50(17.49%) respectively) of AD patients followed by streptococci spp, some bacterial types of Enterobacteriaceae , and others . Since, – in especially - both *Staph .aureus* and streptococci can produce superantigens ,which can activate langerhans cells (LCs) and other cells (monocytes ,macrophages , Tcells , and keratinocytes) in a nonspecific manner , these bacteria are believed to be acting as a key modulators of the inflammatory Cascade in AD skin . (Uehara,1985; Cooper. 1993 ; Strickland , *etal.,* 1999; Sampson ,1999; Hanifin &Chan , 1999; leung , 2000 and Ramirez *, etal.,* 2002).

Many modern studies illustrated the factors responsible for the preferred colonization of atopic skin . The first step in any bacterial infection process is the adherence of the bacterium to hostel (Abeck & Mempel,1998). Mempel, etal,

(1998) studied the role of *Staph.aureus* surface -associated proteins in the attachment to HaCaT keratinocytes and showed that there are several protein involved ,Such as staphylococcal protein A, Coagulase , Clumping factor and fibronectin binding proteins . Other factors whose relevance to the increased colonization of atopic skin with *Staph . aureus* are pH values were shifting toward alkalinity with adherence of *Staph . aureus* to human keratinocytes being highest at PH=7-8(Seidenari & Giusti, 1995), and extracellular lipids of the stratum corneum , the quantitative and qualitative changes in lipid composition could result in diminished antibacterial activity . (Abeck, *etal .,* 1996; Ohnishi, *etal.,* 1999 and Arikawa, *etal.,* 2002).

Our work on antistaphylococcal ability of thirteen antibiotics and revealed that , Vancomycin was the best antibiotic followed by other (Amoxycillin /Clavulanic acid , Bacitracin, Rifampicin, Gentamicin, Cephalothin, Clindamycin, Tetracyclin, Chloramphenicol, Methicillin ,Doxycyclin Hcl, Cotrimoxazole, and Erythromycin).Also , we studied Modes of antibiotic resistance and the results illustrated a high significant incidence of resistance against two , three , four, and five or more of antibiotics ($P<0.001$).

Some of the studied antibiotics were tested by previous studies, while others as Ac, B, Do ,Co, R and Va were not studied in any of atopic dermatitis investigations .

Ring,*etal*. (1996) suggested that as a significant number of *Staph . aureus* are resistant to erythromycin , the antibiotics of choice are pencillinase – resistant pencillins such as flucloxacillin , the oral cephalosporins such as cephalexin and fusidic acid . Systemic antistaphylococcal antibiotics are particularly helpful in the treatment of acute exacerbations of AD due to diffuse *Staph.aureus* infection. (Abeck & Mempel, 1998).

Lever, *etal*. (1988) described that erythromycin and the newer macrolide antibiotics (azithromycin and clarithromycin) are usually beneficial for patients who

are not colonized with a macrolide –resistant *Staph.aureus* strain . However, for macrolide – resistant *Staph aureus* , a pencillinase–resistant penicillin (e.g.,dicloxacillin) may be needed .

Due to the increased risk of bacterial resistance accompanying frequent use of antibiotics, it is important to combine antimicrobial therapy with effective skin care since it is well established that the excoriated inflammed skin of AD predisposes to *Staph. aureus* colonization. (Leung, 2002).Therefore, use of antibiotic therapy must be carried out with a good skin hydration to restore skin barrier function and effective anti-inflammatory therapy to reduce overall skin inflammation and *Staph . aureus* colonization (Matsumoto, *etal* ., 1999).

Exacerbating factors such as food allergens , inhalant allergens , irritants , and emotional triggers should be identified and eliminated because they can alter response to therapy . Since, the major reservoir for *Staph . aureus* is the nose, intranasal antibiotics may be needed to reduce overall skin carriage of *Staph . aureus* (Ramsay, *etal.,*1996, and Abeck &Mempel, 1998)

4-5- *Staph aureus* exotoxin (staphylogen or Staphylogenic Protein (as superantigen)

A technique composed of five steps were used to isolation, purification, identification and Characterization of *Staph . aureus* exotoxin (staphylogen) and all antigens includes : primary screening , primary detection , production assay, purification techniques (precipitation by $(NH_4)_2SO_4$), membranous infiltration (dialysis), and gel filtration Chromatography by Sephadex G -100) and characterization of staphylogen (estimation of lytic activity , kinetic properties, cytotoxicity , antibacterial activity and evaluation of purity and molecular weight by polyacrylamide gel electrophoresis (PAGE7.5%) . Application of all these steps/or techniques yielded a highly purified single band protein of *Staph . aureus* exotoxin (staphylogen) has a molecular weight of (47.315)kd, and eight purified

bands of all *Staph . aureus* 1 antigens having a molecular weights of (549.540 , 305.492 , 234.422 , 121.618 ,58.884, 31.622 ,19.186 and 13.567)Kd.

Previous studies agreed and evidenced with our findings suggested that , a strongly correlation between *Staph.aureus* exotoxin isolated from eczematous lesions and atopic dermatitis such as Hauser, *etal.*(1996) who found that the staphylococcal enterotoxin A , B,C1,C2 , C3 , E , the toxic shock syndrome toxin-1, and perhaps other *Staph.aureus* products to be identified in the future, belong to a class of proteins collectively termed superantigens and /or staphylogen may interact with imunopathogesis of atopic dermatitis .

Abeck and Mempel (1998), also, confirmed our results by findings revealed that *Staph.aureus* induces inflammatory reactions via a range of activities , including protein and toxin secretion . Among these are the *Staph.aureus* superantigens produced by 57-65% of isolated strains , which have been intensively studied during the last years and have been characterized as substances with potent inflammatory and immunological effects. (Leung, *etal.,*1993., and McFadden , *etal.,* 1993).

Breuer , *etal*. (2000) concluded a relationship between severity of skin lesion and sensitization to staphylococcal enterotoxin B(SEB)in adult patients with AD, and showed that 30-60% of *Staph.aureus* strains isolated from AD patients are able to produce exotoxins with superantigenic properties, mostly staphylococcal enterotoxins A,B,C and D (SEA-D)and toxic shock syndrome toxin -1 (TSST-1).

Robinson, *etal*. (1979) isolated the lysostaphin endopeptidase from *Staph. saprophyticus* which has a MW of 25000 d .

OKino ,*etal*. (1998) discovered a novel type of ceramidase of *Pseudomonas aeruginosa* AN17 isolated from the skin of a patient with a topic dermatitis , and purified 83400-fold and its molecular weight was estimated to be 70 Kd on SDS-polyacrylamide gel electrophoresis.

Landhani ,*etal.* (1999) defined two exfoliative toxins produced by *Staph.aureus* ETA and ETB that consist of 242 and 246 amino acids respectively , with a molecular weight of 26, 950 and 27,274 Kd. A controversial study found that these exfoliative toxin are not superantigens (Plano, *etal.,*2000).

Several staphylococcal superantigens have been described of which the staphylococcal enterotoxins (SEs) are the most widely studied . The SEs are a family of structurally related heat-stable proteins of approximately 27 Kd molecular mass comprising several major serological types (A to E) and three newly characterized SEs (types G to I) and more recently a novel 26 Kd type K (Balaban & Rasooly, 2000; Bachert, *etal .,* 2002 and Johansson , 2005).

A superantigen producing *Staph.aureus* Could be isolated from the skin of patient with AD . (Hauser,*etal .,* 1996). It is clear that it will not be easy to assemble supportive evidence for the fulfillment of Koch's postulates . Nevertheless this hypothesis should actively be pursued because some abnormalities found in AD may be explained by the action of superantigens . For example, superantigen presented by Keratinocytes expressing MHC class II molecules may activate Tcells in such a manner that they release type 2 lymphokines such as IL-4 and IL-5 but not the type 1 Lymphokines IL-2 and IFN-γ . (Goodman, *etal .,* 1994).

The evidence that staphylococcal superantigens interact with cells of the immune system in AD by inducing specific antibodies , theoretically also makes superantigenic activation of immune cells possible . In such a case , patients may produce particularly high levels of IgE through polyclonal activation of B cells . A high levels of serum IgE >100 U /ml are common in AD (Hauser,*etal .,*1996) ; this findings confirmed our immunological results –as previously discussed -.

4-6- *In vivo* and histopathological studies

TO confirm our evidence suggestion that *Staph.aureus* produce exotoxin and /or antigenic components that may be responsible to induce eczematous lesions of atopic dermatitis , the *In vivo* and histopathological studies were found very necessary according to the followings :

1- To confirm Koch's postulates that we hypothesized to illustrate atopic dermatitis from bacteriological view . Our *In vivo* results successed to show eczematous –like lesions on mice skin after experimentally infected with staphylogen, all bacterial antigens, and viable cells of *Staph.aureus*.

2- Typical well Known histopathological changes of eczematous lesions of AD patients were seen in our study and same of these histopathological features were shown in histological examination of slices From eczematous –like lesions of mice skin.

3- The correlations between the clinical appearances of eczematous like lesions on mice skin and histopathological changes of all epidermal and dermal layers of skin biopsy indicated that infected of mice experimentally by various infectious agents were highly evidenced that the mice skin lesion were identical to that of AD human skin lesions .

4- The strongly correlations and major identically clinical and histopathological data are for that of *Staph aureus* exotoxin (staphylogen),that may be had a major role in induction and causing of atopic dermatitis according to above informations .

Over the last few decades , a great advancement has been achieved in revealing the inflammatory components of the atopic dermatitis . By histology ,

acute lesions are characterized by disease : non specific acanthosis , para- and hyperkeratosis and spongiosis. Macroscopically , the lesions are characterized by pruritic erythmatous excoriated papules , WHILE THE chronic inflammatory lesions are defined by presence of lichenifecation and dry and fibrotic papules (Leung & Soter, 2001 and Herz, *etal.* ,2002).

The presence of spongiosis and exocytosis are the main histopathological features of AD , particularly , acute and sub acute type . On the other hand , clinical detection of erythmatous , scaly and itchy lesion forms the clinical triangle of AD . From these findings , it is clearly seen that eczematous lesions can be induced by *Staphylococcus aureus* exotoxine and evidenced their role in the pathogenicity of AD . Mean while the addition of topical antibiotic to topical steroid was found by many studies to be more effective in treating AD than steroid alone (Burns , *etal* ; 2004) . The last point confirm our findings .

Many previous studies were successeded in inducing atopic dermatitis in various animal models (BALB/C mice NC/Nga mice, NAO mice ,SCID mice, domestic animals such as dogs , cats , cow, and horses and others such as Finnish reindeer herders and Felids . (Larmi, *etal.,* 1988 ;Mason & Lloyd ,1989 ; Willemse, *etal.,* 1990; Kunkle & Horner , 1992 ; Rosser , *etal.,* 1993; Ermel, *etal.,* 1997 ; Kondo, *etal.,* 1997 ; Matsuda, *etal.,* 1997 ; Olivry, *etal.,* 1997 ; Roosje , *etal.,* 1997 ; Weck, *etal.,* 1997 ;Herz , *etal.,* 1998 ;Hiroi, *etal.,* 1998 ; Lian &Halliwell, 1998 ;Herz , *etal.,* 1999 ; Matsumoto, *etal.,* 1999; Suto, *etal.,* 1999 and Vestergaard, *etal.,* 1999).

Chapter five

Conclusions & Recommendations

5-1 Conclusions :

1- Infantile and child hood groups were recorded a high prevalence 34.7% and 27.5% respectively than adult age group (37.9%). Males were highly affected with AD in age groups (1,4,5) , while females predominated male in groups (2,3). In general, the male: female ratio of infection with AD was (1:1.29) (P< 0.001).

2- Face and face & neck were the commonest sites of predilection of AD lesions (33.1 % and 20%) respectively .

3- A high correlations between family and personal histories were shown with AD.

4- Dry type of skin , sub acute pattern of disease course, and mild to severe degree of severity were predominant forms in AD patients.

5- The values of Hb, RBCs count and basophiles of AD patients were under the normal or control means , while values of ESR, WBCs count , lymphocytes count , eosinophiles and platelets count were higher than normal means . On other hands , values of differential neutrophils , lymphocytes and monocytes were within normal means (P< 0.001) .

6- The study showed a high significant elevation of various types of cluster of differentiation (CD): (CD3 ,CD4 , CD8 , CD19) .Also , all means of concentration of immunoglobulin (Ig) (IgA, IgG , IgM and IgE) and complement components C3 and C4 were increased more than those for control group(P<0.001).

7- AD patients were grouped in to three groups of allergy modes according to concentration of total IgE measured by (two- sites enzyme linked immunosorbent assay (ELISA), in which it was found that mode of allergy (very probable)(IgE> 100 IU) was predominant in 88,8% of various age groups (P<0.001)

8- Fourty environmental (food , fungal , agricultural , chemical and aero-) allergens , and eleven extracted bacterial antigens (as allergens) were tested against specific IgE by using EIA (enzyme immuno assay) technique. We found a very high significant allergens – specific IgE reaction with a high degree mediated a various forms of hypersensitivity in AD patients.

We can arranged various allergens according to high degree of hypersensitivity as follows($p<0.001$):

Sweet veneral grass>rose >sheep s wool >banana >codfish >cotton cultivated >common reed >scale >milk> chicken feathers >onion > cat epithelium >pigeon droppings >silk >chicken >pepper tree >plane tree >dog dander >yolk > cockroach > garlic > wheat > *Candida albicans* >olive > pencilloyl G> horse epithelium > egg white > cow dander >cheddar cheese.

While other allergens were found no statistical significant differences($p>0.05$).

9- *Staph.aureus* exotoxin recorded 100% for the highest degrees of hypersensitivity modes (B,A,H and more) . ($P<0.001$).

10- The results revealed a positive cultures from the total AD patients (eczematous lesions and healthy skin) in 94.4% and 86.36% respectively in total number ranged from 0.02- 92.0 $x10^5$ cell/ cm and 0.11 – 23.0 $x10^3$ cell /cm in the same mentioned sites respectively .

11- *Staphylococcus aureus* was a predominant bacterial agent isolated in 60.48% and 17.48% from the above mentioned sites respectively, followed by other bacterial types in various percentages of isolation .

12- Antistaphylococcal ability of thirteen antibiotics were studied where Vancomycin was found to be the best affective antibiotic according to antibiotics susceptibility test , followed by others. And , a high significant incidence of resistance of *Staph.aureus* against double, three, four and five or more of antibiotics ($P<0.001$)

13- A technique of five steps were used to isolate , purify , identify and characterize the *Staph .aureus* exotoxin (staphylogen / or staphylogenic protein as a superantigen), where its purity and molecular weight were evaluated by using Polyacrylamide gel electrophoresis(PAGE 7.5%) .

14- A high purified single band protein of *Staph .aureus* exotoxin has a molecular weight of 9 47.315) Kd , and eight purified bands of all *Staph.aureus* antigens have a molecular weight ranged from (13.567 – 549.540)Kd.

15- *In vivo* results successed to induce eczematous - like lesions on mice (BALB/C) skin after its experimental infection with staphylogen , all bacterial

antigens and two doses of viable cells and OMPs of *Staph.aureus* ,by using a various infection methods : intradermal , spot and prick technique of injection .

b- Typical well known histopathological changes of eczematous lesions of AD patients were seen in our study and the same histopathological features were shown in histological examination of slices from eczematous like lesions of mice skin.

5-2- Recommendations

1- A study of other epidemiological factors that may modulate(affect) atopic dermatitis such as; using of cosmetics , working status , living habitat, type of cloths ,climate effects , use of drugs ,modes of food intake , exposure to animal house wastes, and others.

2- A study of most relevant blood components such as lymphocytes, monocytes , eosinophiles and basophiles , and their role in the epidermal and dermal layers of the skin of human and other laboratory animals .

3- A study of the relationships between various bacterial types present in eczematous lesions to determine the condition that encourage the survival and the pathogenesis of these bacteria in atopic dermatitis.

4- Use a biotechnology to discover the gene sequences of *Staph aureus* isolated from eczematous lesions , and/ or amino acid sequences of exotoxin/or staphylogen.

5- determine the molecular role of IgE in atopic dermatitis and the mechanisms of allergy and hypersensitivity associated with related allergens / or antigens.

Chapter six

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