Republic of Iraq

Ministry of Higher Education

And Scientific Research

University of Kerbala

College of Science

Department of Biology

Bacteriological and Immunological Determinants of Atopic Dermatitis Patients in Holy Kerbala Province

A Thesis Submitted to the Council of the College of Science at University of Kerbala in Partial Fulfillment of the Requirements for the Master Degree in Biology

By

Zahraa Hadi Al- Wazni

B.Sc. Biology/ University of Kerbala (2017)

Supervised by

Assist prof.Dr.Ali Atia Abid Al Hisnawi **Prof. Dr. Wafaa Sadeq Al Wazni**

April, 2021 A.D Shaeban ,1442 A.H

بسم الله الرحمن الرحيم

قال رب اشرح لي صدري ويسر لي امري واحلل عقدة من لساني يفقهوا قولي

صدق الله العلي العظيم

DEDICATION

To the source of my happiness and strength in this life ,my support, **my Father**.

To the wonderful ,my love ,who prays for me, **my mother**.

To my lovely three brothers and my sweety only one sister

Zahra Hadi Al Wazni

Acknowledgements

In the name of Allah the merciful, lord of the worlds, and prayers and peace be upon the most honorable creatures and messengers Muhammad. I thank Almighty Allah for what He had helped me and made it easy for me throughout the research period so that I can complete it. I would like to begin by thanking **Dr. Wafaa** Sadeq Al-Wazni and **Dr.Ali** Al-Hisnawi, who gave me the opportunity to prepare the research under their supervision; with their support and with the help of Almighty Allah I was able to present this research. I would also like to thank the Department of Biology / College of Science / University of Kerbala, for offering the scientific support,education and facilities. I would also like to mention the Dermatology department at the Al Hussein Educational Hospital in Kerbala province and their valuable help to me, speacially **Dr. Zainab** Najem. Additionly, I would like to extend my gratitude to Atopic dermatitis patients for their full cooperation and presentation of their samples for my study. Finally, I would like to thank my friends **Ruqia** saad, **shaimaa** salim and **Haider** Al- zubaidy for their distinct efforts to help me.

Zahraa Hadi Alwazni

Committee Certification

We certify that we have read this thesis, entitled "**Bacterilogical and Immunological determinants of Atopic Dermatitis patients in holy kerbala province**" and as an examination committee ,examined the student (Zahraa Hadi Al- wazni) on its content, and that in our opinion ; it is adequate for the partial fulfillment of the requirements for the Degree of Master of Scince of Biology.

Signature:
Name: Dr. Ali M.Almohana
Scientific degree: Professor
Address: College of Medicine
University of Kufa

Date: / /

Signature:

Name: Wejdan Ridha Tajaldeen

Scientific degree: Professor

Address: College of Scince

University of Babylon

Date: / /

Signature:
Name: Dr. Hiyam AbdulRidha kareem AlAwad
Scientific degree: Assist professor

Address: College of education for pure Scince
University of Kerbala

Date: / /

Signature:

Name: Dr. Wafaa S. Mohsin Al-wazni

Scientific degree: professor

Address: College of Science

University of Kerbala.

Date: / /

Signature:

Name: **Dr. Ali A.Abid Al Hisnawi**

Scientific degree: Assist professor

Address: College of Scince

University of Kerbala.

Date: / /

Approved for the council of college

Signature
Name: Dr.Jasem Hanoon Hashim Al- Awadi
Scientific degree: Assist professor
Address: Dean of College of Science / University of Kerbala.
Date: / /2021

Supervisors Certification

We certify that this thesis entitled "**Bacterilogical and Immunological determinants of Atopic Dermatitis patients in holy kerbala province**" was prepared under our supervision at the department of Biology,College of Science, University of Kerbala, as a partial fulfillment of the requirements for the Degree of Master of Science in Biology.

Member: Supervisor

Signature:

Name: Ali Ateia Abid Al Hisnawi

Title: Assist.professor

Address: College of Science/ University of Kerbala.

Member: Supervisor

Signature:

Name: Dr. Wafaa Sadeq Mohsin Al-Wazni

Title: professor

Address: College of Scince/ University of Kerbala.

Chair of the Biology Department

In view of the available recommendation, I forward this thesis for debate by the examining committee.

Head of Department

Signature:

Name: Assist. Prof. Dr. Khalid Ali Hussein

College of Science / University of Kerbala

Abstract

Atopic dermatitis (AD) is a multifaceted disease that results from a complex relationship between genetic and environmental factors. Both these variables will form the role of the skin barrier infection and the immunological response of predisposed patients. The current study was conducted to evaluate the correlation between serum level of some immunological parameters such as Interleukin-5 (IL-5), Interleukin-13 (IL-13) and immunoglobulin E (IgE) with skin bacterial infection and severity of AD in patients.

Eighty cases including 50 patients (20 males and 30 females) with history of AD that diagnosed according to the clinical symptoms and by examining physicians were enrolled in the present study. In addition 30 healthy cases were selected as a control group in Kerbala province, during the period from December 2019 to July 2020. The procedures of present study were performed at the laboratories of Al- Hussein Teaching Hospital, when distinguished of AD according to the age and gender. Data obtained from the current study showed that the percentage of AD in females was (60%) more than in males (40%), in addition the highest percentage of infection with AD (60%) was in the patients of 5-15 age category while the lowest infection percentage (18%) was in the 26-35 age group compared to other age group.

When cultured 130 skin swab samples from 50 patients with AD (50 sample from lesional loci and 50 sample from surrounding area of lesional loci) and 30 sample from control group , that diagnosed by biochemical test ,Vitek system, and PCR technique. 31 isolates (91%) of Gram positive bacteria represented by *Staphylococcus. aureus* (70.5%) were the commonest isolated genera followed by Bacillus spp (8.8%), *Kokuria rhizophila*(5.9%), *Staphylococcus. epidermidis*(2.9%), *Staphylococcus heamolyticus*(2.9%), but only 3 isolates (8.8%) were Gram negative bacteria that represented by *Pseudomonas stutzeri* from lesional loci of AD patients. In addition Gram positive bacteria included *S. aureus* , *S .epidermidis* and *S. heamolyticus* were

isolated from surrounding loci of AD lesional area in high percentage compared to Gram negative bacteria, one isolates (3%) that represented by *P .stutzeri*. Furthermore the profile of bacterial which is isolated from skin of control group were represented by low percentage of *S. aureus* 4 isolates (20%) with high percentage of normal flora 16 isolates (80%) *S. epidermidis*.

Blood samples were collected also form the patients to determine the serum level of IgE, IL-5 and IL-13 in all studied groups .The results revealed a significant increase in the patient groups (378.2) (13.3) and (23.6) respectively compared to the control group that reach to (65.1) (2.2) and (2.06).

In addition results of white blood cells count showed a significant increase ($P < 0.05$) in patients with AD in the females (8.48) and males (7.80) compared to males (6.86) and females (7.10) in the control group respectively. And an a significant variant of differential WBCs count ,the mean of neutrophils cell showed significant increase in females (51.72) compared to males (46.21).In addition the lymphocytes cell were significant increase in males (37.42) in compared to females (36.02) but is non-significant increase in males compared to control group. The eosinophils and basophils show significant increase ($P < 0.05$) in the both sex compared to control group .

It can be concluded from these results that the relationship between the variance of certain immunological parameters in the AD patients with the disease and development of bacterial infection .

List of contents

List of contents

List of Tables

List of Figures

List of abbreviations

ACD	Allergic contact dermatitis
AD	Atopic Dermatitis
AMPs	Antimicrobial peptides
CBC	Complete blood count
CCL17	Chemokine (c-cmotif) linked 17
CD	Cluster of differentiation or cluster of designation
DCs	Dendritic cells
DNA	Deoxyribonucleic acid
ECP	Eosinophil cationic protein
EDTA	Ethylene diamine tetra acetic acid
ELISA	The enzyme-linked immunosorbent assay
FA	Food allergy
Fcr	Fragment crystallizable region
FFAs	Free fatty acids
FLG	Filaggrin gene
GM-CSF	Granulocyte macrophage colony-stimulating factor
GWAS	Genome-wide association study
H2O2	Hydrogen peroxide
HRP	Horse radish peroxidase
IFM ϒ	Interferon gamma
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IL13	Interleukin 13
IL5	Interleukin 5

ILCs	Innate lymphoid cells
iNKT	Invariant natural killer T
IV	Ichthyosis vulgaris
LCs	Langerhans cells
MDC	Macrophage –derived chemokine
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSA	Mnnitol salt agar
NaCl	Sodium chloride
OD	Optical density
PCR	Polymerase chain reaction
PH	Power of hydrogen
RBC	Red blood cell
Rpm	Rapid per minutes
rRNA	Ribosomal –Ribonucleic acid
RT	Room temperature
SC	Stratum corneum
SD	Standard deviation
SEB	staphylococcal enterotoxin B
SEE	Staphylococcal enterotoxin E
SsAgs	Staphylococcal superantigens
TARC	Thymus –and activation –regulated chemokine
TBE	Tris borate EDTA
Th17	T helper 17
Th2	T helper 2
TM	Melting temperature
TNF-α	Tumor necrosis factor α

TSLP	Thymic stromal lymphopoietin
TSST	Toxic shock syndrome toxin
WBC	White blood cell

Chapter One

Introduction and Literature Review

1.1 Introduction

Atopic dermatitis is the most common chronic inflammatory skin disease with the genetic predisposition, epidermal barrier disruption and dysregulation of the immune system (Kim *et al*., 2019). In addition, the inherited or acquired filaggrin deficiency has been described to essentially contribute to the pathogenesis of AD (Cabanillas and Novak, 2016).

The skin barrier defects, atopic immune dysregulation, environment facilitate, allergen sensitization, and the development of other IgE-mediated allergic diseases in a process called the atopic march, in which AD is also associated epidemiologically with several autoimmune diseases showing auto reactive IgE secretion (Pellefigues, 2020).One current hypothesis on potential biological mechanisms for the atopic march revolves around defective skin barrier function, this defective is thought to allow both epidermal water loss and penetration by high molecular weight structures such as allergens, bacteria and viruses (Dharmage *et al*., 2014).

The pathogenesis of AD may include disrupted epidermal barrier function immunodysregulation, and IgE-mediated sensitization to food and environmental allergens, AD is also part of a process called the atopic march, that lead to progression from AD to allergic rhinitis and asthma, This has been supported by multiple cross-sectional and longitudinal studies and experimental data (Zheng *et al*., 2011; Lee and Lee, 2014).

Genetically inherited mutations affecting on barrier function in combination with acquired environmental stressors are currently proposed to result in increased penetration of allergens, This promotes a change in Thelper-2 cells (Th2) that further worsens the skin barrier providing

protection from infection (Marsella *et al*., 2011).Two complex and complementary, effective techniques, namely the prevention of pathogen invasion and the increase of innate and/or adaptive immune responses after infection (Biedermann *et al*., 2015). The patients with AD exhibit both, impaired skin barrier function and defects in skin innate immunity, therefore AD patients frequently develop skin infections which contribute to the pathogenesis and the course of their chronic inflammatory skin condition (Mrabet and Maurer, 2011).

Both CD4+ and CD8+ T-cells bearing the cutaneous-lymphocyte-associated antigen represent activated memory/effector T-cell subsets and induce IgE, mainly via IL-13, and prolong eosinophil lifespan, mainly via IL-5.Dysregulated apoptosis in skin-homing T-cells and keratinocytes contributes to the elicitation and progress of AD (Akdis *et al*., 2000). IL-13 is a cytokine derived from T-cells that has many functions, such as the induction of IgE synthesis, besides having a direct role in AD when developed in the skin and causing a chronic inflammatory phenotype characterized by xerosis and pruritic eczematous lesion (Zheng *et al*., 2009).

An imbalance of the adaptive immune system mediated by various T cells plays a pivotal role in the pathogenesis of AD, traditionally sustained exposure of pathogens tailors immune responses and drives the development of specialized T-helper cells cytokine environment (Wang and Ning, 2015).The immune system assists the host to eliminating harmful or allergenic substances that reach via mucosal surfaces, to recognize and destroy microbes (such as bacteria), the host employs both

innate and adaptive mechanisms (Chaplin,2010).The increase understanding of the T-cell biology has refreshed the roles of classical Th2 responses and regulatory T-cells in the development of AD skin that showing dominance of IL-13 pathways and it is directly involved in the regulation of IgE and has been associated with atopy (Hussein *et al*., 2011). *S. aureus* was the most prevalent bacteria on skin of AD patients compared to control skin, and positively correlated to severity of disease but on other hand, the affected skin sites were more dominated by *S. aureus* than unaffected sites particularly inflamed areas and during a flare the abundance increased dramatically in untreated patients. Also *S. epidermidis* and *S. heamolyticus* were increased on involved sites (Bjerre *et al*., 2017).So when, skin becomes more vulnerable to bacterial inflammation that raises the severity of AD. Therefore, the present study was designed to evaluate the relationship between the levels of some immunological parameters in AD patients with the disease and increased the chance of skin bacterial infection in those patients. This aim will be accomplished through these objectives:

1. Isolation and identification of the aerobic bacterial agent of skin lesion in AD patients.

2. Diagnosed the isolated bacteria biochemically and by PCR technique.

3. Measuring the level of IL-5, IL-13 and IgE in AD patients and compared with healthy control group.

4. Evolution the relationship between the percentage of isolated bacteria and variation in some immunological parameters.

1.2 Literature review

1.2.1 Definition of Atopic Dermatitis

Atopic dermatitis (atopic eczema) is a widespread inflammatory skin condition characterized by defects in both skin barrier structures and immune response alternations, so that the major cause for structural abnormalities in the epidermidis associated with immune dysregulation in this disease (Salava and Lauerma, 2014). Comorbidities of AD include allergies (food allergy, asthma, allergic rhinitis, allergic conjunctivitis, and eosinophilic esophagitis), implying both dermal and systemic immune response, the international eczema council discovered a robust pattern of immune activation in peripheral blood, as well as an increased risk of skin and systemic infections (Brunner *et al*., 2017).

There is a regular association between AD with other atopic and allergic conditions including asthma and rhinitis, some times in a progression known as the atopic march (Dharmage *et al*., 2014). In the understanding of the eczema pathogenesis, later investigations the role of filaggrin gene (FLG) mutations have identified a series of important associations to the atopic disease phenotypes, including atopic asthma and allergic rhinitis (Brown and McLean, 2012).

Atopic eczema is the most common childhood inflammatory skin disease, affecting 15-20 percent of children at any time in the world **(**Sathishkumar and Moss,2016). In addition to the symptoms of intractable itching, skin injury, pain, loss of sleep and the social stigma of a noticeable skin condition, the cause of atopic eczema is unclear, although a genetic predisposition and a combination of allergic and non-allergic factors seem to be significant in determining the expression of the disease **(**Hoare *et al*., 2000). AD is a non-contagious

inflammatory, chronically ,relapsing, and sever pruritic skin disease often occurring in families with atopic diseases such as (AD ,bronchial asthma and/or allergic rhinitis-conjunctivitis), AD is an eosinophilic/spongiotic inflammation of the skin with characteristic age-dependent distribution patterns and morphology of lesions, accompanied by 2–5% prevalence in young adults and up to 20% in children, AD is one of the most common skin diseases (Wollenberg *et al*., 2016).

The release of type 2 cytokines from activated Th2 cells causes the majority of inflammatory responses seen in allergic diseases like AD disease, and the primary characteristics of allergic reactions include the release of IgE, increased vascular permeability and recruitment of effector cells like eosinophils, basophils, and mast cells (Pulendran and Artis, 2012). Type 2 cytokines are not only produced by Th2 cells; Th9 cells (follicular T helper cells) and inflammatory cells also contribute to the secretion of type 2 cytokines, independent of T-cells, invariant natural killer T-cells produce large quantities of cytokines and cause airway inflammation (Liang *et al*., 2012).

AD is a chronic and pruritic immune-mediated inflammatory dermatitis characterized by a Th2 immune response phenotype and may be associated with systemic inflammation (Gooderham *et al*., 2018). Inflammatory dendritic cells (DCs), macrophages, and eosinophils infiltrate the lesions, resulting in chronic AD, furthermore, pruritus caused by AD disease reduces patients' quality of life and increases their susceptibility to microbial colonization, such as *S. aureus* infections, which is why AD disease is still a major health concern in many countries today, the clinical signs of itching, facial and extensor eczema in babies and toddlers, flexural eczema in adults, and dermatitis chronicity are used to diagnose AD (Cevikbas and Steinhoff, 2012).An increase in serum IgE as well as

Th2 immune responses with increased IL-4, IL-5, IL-10, and IL-13 are the most commonly cited explanations for this allergic disease (Wollenberg and Feichtner, 2013).

1.2.2 Types of Atopic Dermatitis

Atopic dermatitis can be classified into two types extrinsic and intrinsic forms. So that, extrinsic or allergic AD shows high total serum IgE levels and the presence of particular IgE for environmental and food allergens, normal IgE is characterized by intrinsic or non-allergic AD and the clinical characteristics include relative late onset, milder severity, no ichthyosis, palmar hyperlinearity (Tokura, 2010). Many studies have showed variations between extrinsic and intrinsic AD in different aspects of pathophysiology (choi *et al*., 2003; Suarez *et al*., 2013). Immunologically, surface expression of the high and low affinity of IgE receptor is elevated in monocytes from patients with extrinsic AD, but serum IL-13 levels are substantially increased in AD patients and neutrophil expression is comparably increased in both forms (Raap *et al*., 2006).

The outermost layer of mammalian skin, stratum corneum SC of the epidermis, consists of piles of dead corneocytes that are the end-products of terminal differentiation of epidermal keratinocytes, so that SC performs a crucial barrier function of epidermis, Langerhans cells, when activated, extend their dendrites through tight junctions just beneath the SC to capture external antigens. Recently, knowledge of the biology of corneocytes (‘corneobiology’) has progressed rapidly and many key factors that modulate its barrier function have been identified and characterized (Matsui and Amagai, 2015). According to several studies, a faulty of skin barrier serves as a site for allergic reactions to antigens and bacterial super antigen colonization, this causes systemic Th2 immunity, which

makes patients more susceptible to allergic nasal reactions and encourages airway hyperreactivity, Pathological inflammation in the skin, and percutaneous sensitization to allergens thus, the most novel treatment strategies seek to target specific aspects of the skin barrier or cutaneous inflammation (Bantz *et al*., 2014; Tsakok *et al*., 2019). Also other study finding that a filaggrin gene mutations in a high percentage of patients with AD, together with an older finding of ceramide reduction in the stratum corneum, have further suggested the presences in extrinsic AD of skin barrier damage (Henderson *et al*., 2008).

On the other hand, eczema is an inflammatory skin condition which can be caused by different causes that are intrinsic and extrinsic, a common form of eczema, chronic generalized eczema, is characterized by multi-site lesions, complex etiology, frequent recurrence, and extreme pruritus, the disease dramatically decreases the patients quality of life and puts a severe economic burden on patients (Li *et al*., 2016).

Extreme AD is generally diffuse, primarily affecting the face, neck, hand, and flexures, although it can affect to some degree all regions of the body, inflammatory versus lichenoid patients with the inflammatory pattern are red in color, the skin displays diffuse erythema with mostly acute, exudative and crusted eczematous lesions, often followed by profuse scaling (Hello *et al*., 2016). Medical evidence has established that AD is a significant risk factor for manual eczema, contact allergy was also confirmed to be an important risk factor for hand eczema and the risk was associated with extreme contact allergy eczema (Bryld *et al* ., 2003). In terms of exposure to water, detergents, additives, and hand washing, the proportion of cases and controls in jobs with a high risk of hand eczema was comparable (Nyren *et al*., 2005). The presumption of adult AD is often posed by

persistent hand eczema in adults, atopic chronic hand eczema's clinical appearance is not necessarily the same medical morphological forms: acute persistent dyshidrotic eczema, a chronic type of irritant contact dermatitis, and chronic dry fingertip dermatitis are differentiated (Seghers *et al*., 2014).

1.2.3 Distribution Patterns of Atopic Dermatitis

Atopic Dermatitis is a chronic inflammatory skin disorder, regardless of the age of the patient, mild to extreme erythema, scaling, and excoriation are typically present that indicate the severe itch, during the lifetime of the patient, the distribution pattern of skin lesions varies from more generalized eruptions of oozing and crusted lesions to the adult distribution pattern of flexural eczema with lichenification and scaly, xerotic, dry uninvolved skin, as seen in **(**Wollenberg and Bieber, 2000).

The clinical symptoms of AD differ with age and it is also possible to distinguish three stages, the first eczematous lesions typically form on the cheeks and the scalp in infancy as shown in figure (1-1), crusted erosions are caused by scratching, which sometimes begins a few weeks later (Shaw *et al*., 2011).

Lesions include flexures, nape, and the dorsal components of the limbs throughout infancy, in puberty and adulthood, the last stage is lichenified plaques that affect the flexures of the head and neck, itching, which continues during the day and worsens at night, causes sleep deprivation at each stage and greatly impairs the quality of life of the patient. AD is characterized by a chronic, relapsing type of skin inflammation, a disruption of the function of the epidermal barrier function that culminates in dry skin and IgE _ mediated sensitization to food and environmental allergens (Bieber, 2008; James *et al* ., 2011).

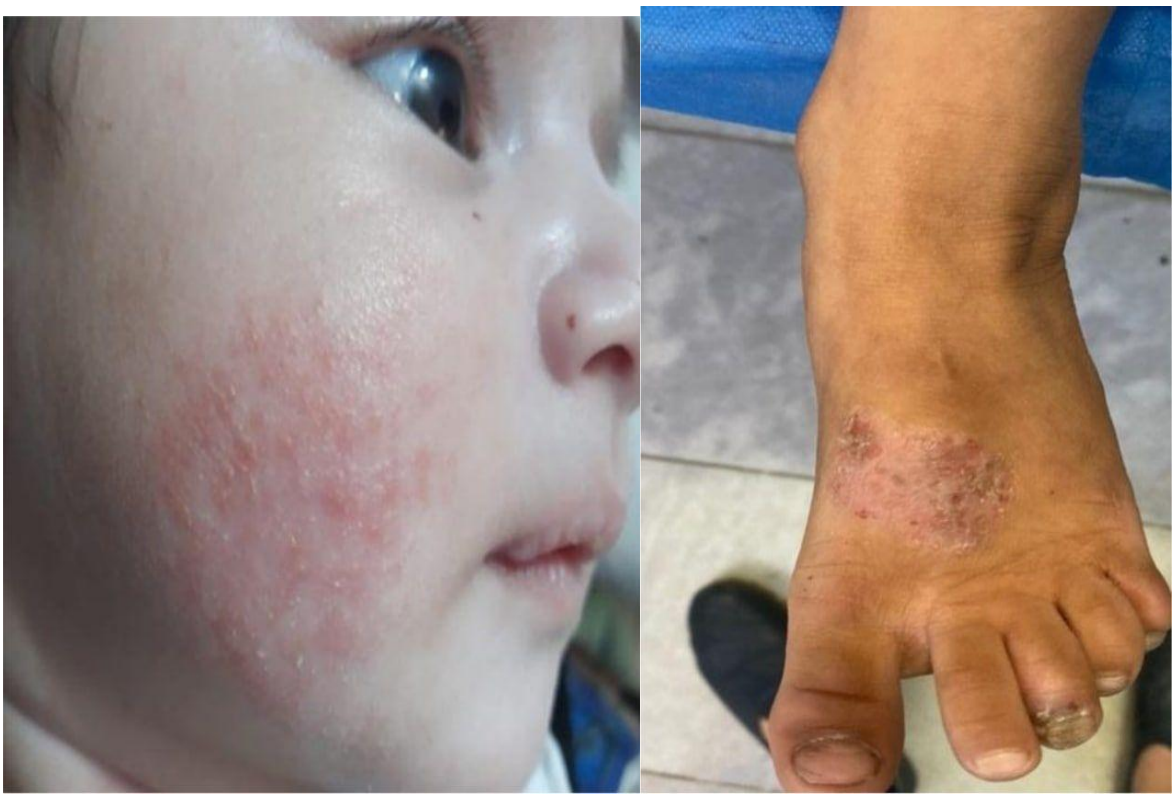


Figure (1-1) :Distribution of AD in children (Zina, 2021)

1.2.4 Pathophysiology

Atopic dermatitis pathophysiology is multifaceted, involving a complex interaction of many variables, including biology, climate, and dysregulated immune pathways (Deleanu and Nedelea, 2019). Genetic factors, as well as gene-environmental interactions, play a pivotal role in the pathogenesis of AD disease, several genes linked to skin barrier dysfunction, proteolytic activity, and the immune system which have been linked to AD disease, suggesting that they may play a role in the etiology of the dysfunctional skin barrier and immunological abnormalities seen in AD skin **(**Sacco and Milner, 2019).

Severe pruritus, xerosis, increased transepidermal water loss, normally distributed eczema lesions in flexural areas, and frequent cutaneous bacterial infections are all symptoms of AD, this appears to be aided by a weakened skin barrier (Jinnestal *et al*., 2014). AD is a common skin disorder and continues to increase in prevalence worldwide, several studies on AD pathogenesis and associated disorders have contributed to skin barrier dysfunction and to the adverse effect of FLG mutations on barrier function have raised the question of whether these barrier defects allow secondary changes in immunologic response that mediate the development of AD (Eichenfield *et al*., 2012).

The epidermidis (outer layer of skin) is made up of four layers: a proliferating keratinocyte basal layer, a spinous layer, a granular layer, and mortar framework, the stratum corneum, which consists of a dense layer of corneocytes arising from the differentials of keratinocytes in the epidermal layer below, the right junctions that limit the movement of large molecules or pathogens through the skin keep these cells together (Van Smeden *et al*., 2014).

The stratum corneum contains lipids such as ceramides, free fatty acids FFAs, and cholesterol, in patients with AD disease, there is a general decrease in lipid levels, particularly in ceramide content and ceramide chain duration, which is linked to the severity of the disease (Janssen *et al* ., 2012).The Th2 immune response activation, with IL-4, IL-5, and IL-10 inhibiting delayed hypersensitivity, is one of the early immunological events observed in the development of atopic lesions interferon (IFN) growth is inhibited by IL-4, urticarial lesions are also common in the early stages of AD disease, as a result of Th2 hyperreactivity(Otsuka *et al* .,2017).

These immunological changes have the effect of reducing the development of antimicrobial peptides (AMPs) (cathelicidin) and β-defensins 2 and 3 in particular, as shown in Figure (1-2), owing to this lack of production of AMPs specifically (cathelicidin) and β-defensins 2 and 3, atopics may be predisposed to widespread skin infections, atopics may be predisposed to widespread skin infections due to viruses (herpes, molluscum, and vaccinia) and bacteria, especially bacteria, due to this loss of AMPs production (*Staphylococcus*) (James *et al* ., 2011) .

The immune response to the production of Th2 cytokine is further distorted by epicutaneous exposure to Staphylococcal superantigens, which generate IgE antibodies in AD patients, explaining the connection between staphylococcal infection and AD exacerbations, staphylococcal superantigens such as enterotoxin B (SEB) staphylococcal, enterotoxin E (SEE) staphylococcal and toxic shock syndrome toxin (TSST-1) induce a deep decrease in T-cell steroid responsiveness of T- cells, another factor related to staphylococcal skin infection or invasion and AD flare-ups is this, chronic cutaneous inflammation is characterized by Th1 cytokines, despite the fact that AD is a Th2-mediated disease, this helps to understand why chronic AD looks like other chronic dermatoses histologically, patients with AD disease have monocytes in their peripheral blood (James *et al* ., 2011).

During disease flares, the percentage of *S.aureus* sequences was higher than at baseline or post-treatment, and was linked to the disease's worsening nature, during flares, skin representation of commensal *S. epidermidis* improved significantly (kong *et al* ., 2012).

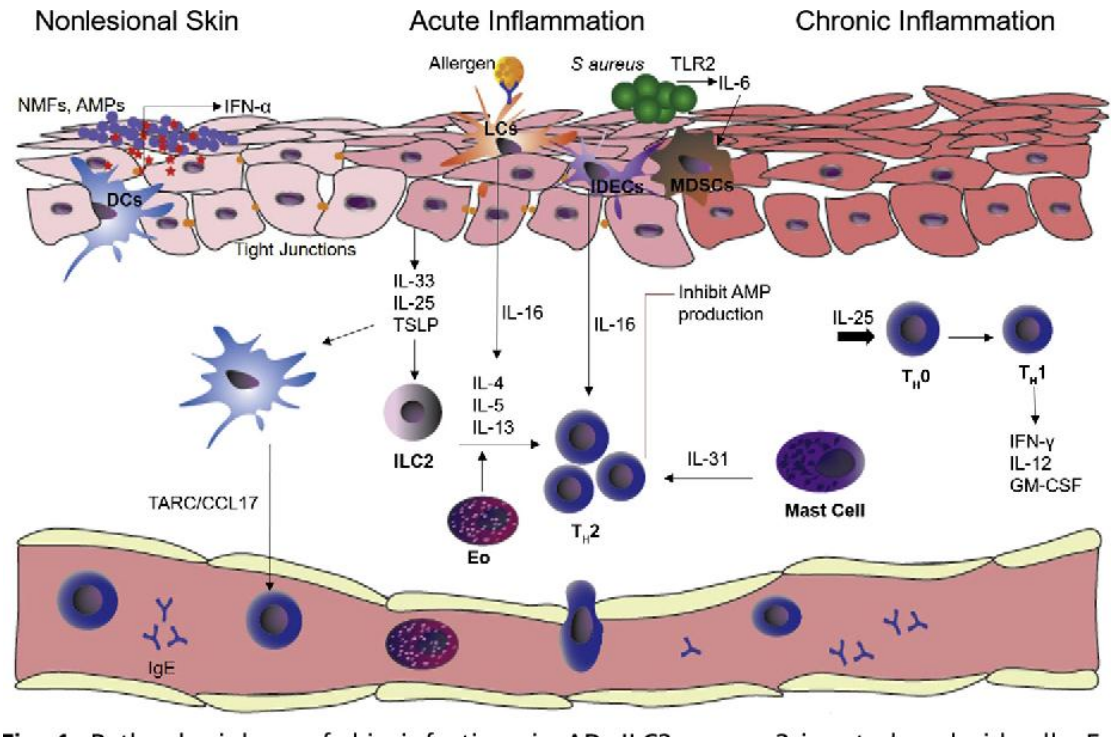


Figure (1-2): Pathophysiology of AD (Liu *et al*., 2011)

1.2.5 Epidemiology

Atopic dermatitis (eczema) is a chronic pruritic, inflammatory skin condition with a global prevalence of affecting 11–20% of children and 5–10% of adults (Cork *et al*., 2020).Clear epidemiological similarities exist between asthma and AD, importantly, the first manifestation of atopic diathesis, which occurs in genetically predisposed people and often involves asthma and allergic rhinitis, is often AD later in childhood, up to 80% of children with AD can develop allergic rhinitis or asthma (Eichenfield *et al*., 2003). Among children and adults 20 % and 3% , respectively are affected by AD, according to recent data, its prevalence is still increasing, especially in low-income countries the signs of AD disease usually

appear early in life, and they are also accompanied by other allergic conditions such as asthma or allergic rhinitis (Bonamonte *et al*., 2019). Age category (3-13 years) give the highest AD disease possibility 77.3% under consideration compared with control group (Zina ,2021).

Individuals impacted by AD generally have genetically defined risk factors that influence the function of the skin barrier or the immune system genetic mutations alone, however, may not be enough to cause clinical manifestations of AD, and it is merely the association of a defective epidermal barrier with harmful effects of environmental agents in genetically predisposed individuals that contributes to the disease's growth (Nutten, 2015).

Epidemiological research indicating that AD is more prevalent in affluent households, small family size relationships, increased prevalence in migrant communities, and increasing disease prevalence all strongly indicate a significant role for the environment in deciding the expression of the disease, future study gaps include gene-environment interaction assessment, better studies of the natural history of AD, and better clinical trials that answer questions that are important to physicians and their patients (Williams, 2000).

AD is one of the most common skin condition and affect percentage 5-20% of infants with a lower incidence in adulthood in the worldwide (Carroll *et al*., 2005).Around 70 percent of individual relapses of this disease occur before puberty, while 25 percent of people experience the signs and relapses of this disease after puberty, also around 60% of children who are inflicted with this disease at the beginning of birth are susceptible to one or more allergens, whereas children with late onset of this disease are often less susceptible (Thomsen, 2014).

AD is a complex condition induced by the combination of many genetic and environmental factors, particularly in patients with severe disease (Sacotte and Silverberg, 2018).The impact is not just an itchy rash, but also the secondary effects, particularly disturbed sleep, on the patient's psychological well-being and their jobs, in order to provide a holistic approach for health care practitioners to the treatment of atopic dermatitis that is difficult to treat, described as atopic dermatitis that appears to be unresponsive to simple moisturizers and mild potency and topical corticosteroids (Arkwright *et al*., 2013).

AD management poses a clinical challenge and frequently includes topical and systemic treatment combinations, the most efficient anti-inflammatory treatment for severe AD is topical glucocorticoids and topical calcineurin antagonists, which are used for exacerbation control, more recently, for preventive therapy in selected cases, topical corticosteroids remain the central treatment, tacrolimus and pimecrolimus topical calcineurin inhibitors are favored at some sites, an choice for extreme refractory cases is systemic anti-inflammatory therapy, microbial invasion and super infection lead to the exacerbation of the disease and thus justify additional antimicrobial / antiseptic care (Darsow *et al* ., 2013).

1.2.6 Predisposing Factors Associated with Atopic Dermatitis

The history of family in atopic disease is regarded as a risk factor for peoples, along with an increased risk of AD during breastfeeding, no correlation with certain variables involved in the hygiene hypothesis was found, this means that breastfeeding is not recommended for the prevention of AD (Mailhol *et al*., 2009) .

Food allergy has been shown in some studies to be a strong risk factor for the development of other late childhood allergic diseases, food allergy comorbidity lowers the age of onset of AD disease (Kijama *et al*., 2013). General, the skin reflects of body's interface with the world around it therefore, it is subject to multiple insults, serving as inflammation causes, these involve both irritants and allergenic substances, These factors activate the scratching induced by the release of proinflammatory cytokines from atopic keratinocytes to initiate and maintain the inflammatory cascade initiated by atopic keratinocytes release proinflammatory cytokines, Stress has been shown to cause immunologic alterations, and when combined with scratching, it can exacerbate AD disease (Leung and Boguniewicz, 2004).

AD is a complex disease with multiple causes and complex mechanistic pathways according to age of onset, severity of the illness, ethnic modifiers, response to therapy and triggers, AD severity seems to be a risk factor for associated food allergy FA , results from population-based, birth and patient cohorts show that early-onset and severe AD parental history of asthma, and multiple sensitizations are risk factors leading to the atopic march and the development of asthma (Amat *et al*., 2018).

In general several studies accepted that most babies and young children with early atopic eczema in later childhood will develop asthma, a more complicated relationship between early eczema and asthma is indicated (Vander Hulst, 2007; Pyun, 2015). In patients with AD, this may be associated with a greater risk of sensitization to topical agents, including topical treatment, long-term exposure to allergens has been shown to be a risk factor in allergic contact dermatitis ACD in AD, in order to reduce the risk of irritation and sensitization, attention should be

paid to anything that comes into contact with the skin, especially the topical formulations used to treat AD (Mailhol *et al*., 2009).

AD is a significant risk factor for the development of asthma, with an improved odds ratio in children with AD relative to children without AD in some longitudinal studies, and asthma occurs in around 30 percent of patients with AD. Atopic sensitization patients there is a greater chance of advancing in the atopic march towards asthma along with eczema, early onset and severity of AD are the key risk factors for asthma development and persistence (Spergel, 2010).

In AD patients, the skin barrier is considered to be compromised, both in acute eczematous lesions and in clinically unaffected skin lesions, a hereditary predisposition to produce high levels of stratum corneum chymotryptic enzyme will first compromise skin barrier function, corneodesmosomes are prematurely broken down by this protease enzyme, resulting in epidermal barrier impairment. Stratum corneum chymotryptic enzyme production may be further increased and epidermal barrier function weakened by the addition of environmental interactions, such as washing with soap and detergents, or long-term application of topical corticosteroids, exogenous proteases from house dust mites and *S. aureus* can also cause damage to the epidermal barrier, one or more of these combinations of factors As a result, the risk of allergen penetration and subsequent inflammatory reaction increases, leading to disease exacerbation (Cork *et al* ., 2006; Brough *et al*., 2020).

Filaggrin gene encoding FLG mutations have been recognized as the cause of ichthyosis vulgaris (IV) and have been shown to be important predisposing factors for AD (Nomura *et al*., 2008).

The confirmed discovery as a commonly replicated major risk factor for eczema of loss-of-function mutations in the structural protein filaggrin sheds new light on disease mechanisms in eczema, a disorder that was often known to be etiopathogenesis, which was mostly immunological, the results of the FLG mutation are consistent with a proposed unifying theory for eczema pathogenesis that involves a heritable epithelial barrier deficiency, decreased epidermal defense mechanisms for allergens and microbes, polarized Th2 lymphocyte responses, and chronic inflammation, including autoimmune disease (ORegan *et al*., 2008).

Changes in skin pH in AD, especially with regard to the role of the skin barrier and *S. aureus* colonization, proven evidence indicates that, as a result of reductions in filaggrin proteolysis and sweat secretion, the impaired release of proton donors, such as amino acids and lactic acid to the stratum corneum in AD disease is impaired, furthermore, an impaired formation of since an acidic pH is needed for both lipid organization and lipid metabolism in the stratum corneum, these changes can contribute to the disruption of skin barrier function observed in atopic dermatitis (Rippke *et al*., 2004).

1.2.7 Immune Response of Atopic Dermatitis

1.2.7.1 Innate Immunity

In AD patients, the combination of a genetic predisposition for skin barrier dysfunction and dysfunctional innate and adaptive immune responses leads to a higher frequency of bacterial and viral skin infections, the innate immune system quickly mobilizes an unspecific, standardized first-line defense against different pathogens (Wollenberg *et al*., 2011). Skin inflammation is linked to cutaneous expansion of IL-5-producing type 2 innate lymphoid cells, which occurs independently of adaptive immunity, furthermore, when compared to control

subjects, people with filaggrin mutations have a higher frequency of type 2 innate lymphoid cells in their skin (Saunders *et al*., 2016).

Some of the innate immune defects observed in atopic dermatitis are consider as primary defects, such as epithelial barrier defects and defects in signaling or expression of innate receptors, others may be secondary to the effects of the adaptive immune response, for example, deficiencies in antimicrobial peptides may be due to the overexpression of Th2 cytokines such as IL-13(Niebuhr and Werfel, 2010) .

Th2 cytokine producing invariant natural killer T (iNKT) cells, innate lymphoid cells (ILCs), and Th17/Th22 cytokine producing innate cells – (iNKT) cells and natural killer (NK)like cells which can all play a role in innate immune modulation in AD disease, As a result, early control of innate immune responses in AD, before adaptive immune responses by traditional T and B cells, which perpetuate chronic skin inflammation, may be sufficient to relieve acute exacerbations of AD (Park *et al*., 2013).

1.2.7.2 Adaptive Immunity

A central feature in AD immunopathogenesis is dysregulation of Th2 cells and type 2 innate lymphoid cells, which leads to a robust increase in type 2 immune cytokines, including interleukin IL-4, IL-13, and IL-31 (Kim and Leung, 2019). Homing memory skin T-cells play an important role in the pathogenesis of AD disease due to immune dysregulation, as a result, immune dysregulation with predominant Th2 cytokines, such as IL-5 and IL-13, has been proposed as a pathogenesis for AD disease, because Th2 and Th22 responses drive acute AD lesions, a Th2/Th22 polarized environment has been found in AD, while chronic lesions are driven by a Th1 response (Moy *et al*., 2015).

A reported study found that AD is caused by a distorted Th2 immune response, with a Th1:Th2 ratio of 0.09 in chronic AD, Th2 cells accounted for the majority of CD3 and Tcells in biopsy specimens from chronic AD lesions (64 percent), followed by Th17 (30.4 percent), Th22 (3.3 percent), and Th1 cells (4.8 percent) (Moy *et al*., 2015). Th2 cytokines (IL-5 and IL-13) have a permissive impact on microbial invasion and epidermal barrier destruction by inhibiting antimicrobial peptide (AMP) formation, decreasing lipid production in the stratum corneum, and inducing spongiosis (Noda *et al*., 2015).

Keratinocytes are stimulated to express thymic stromal lymphopoietin (TSLP) by IL-4 and IL-13, which serves as a link between barrier defect and Th2 polarization, in transgenic mice, excessive IL-4 causes AD-like lesions, whereas IL-5 attracts eosinophils to chronic AD lesions (Yamanka and Mizutani, 2011).

Since, AD is associated with allergen sensitization, elevated serum IgE and increased of Th2 cytokines (IL-4,IL-5 and IL-13) in both unaffected skin and skin lesion of AD, candidate gene studies for AD have focused on the Th2 pathway indeed, the genome-wide association study (GWAS) assays have repeatedly identified AD genetic risk locity around Th2 genes regions at 5q31 so that genetic variants of genes in the Th2 signaling pathway including IL-5and IL-13 receptor are positive associated with AD (Barnes, 2010) .

1.2.7.3Role of Interleukin 5

Interleukin 5 (IL-5) is produced in lymphocytes, mast cells, eosinophil, and airway smooth muscle and epithelial cells, and is primarily responsible for the maturation and release of eosinophil in the bone marrow, in humans, it is a very selective cytokine because only eosinophil and basophils possess a type-1 cytokine

receptor for IL-5 with a specific α-subunit and the βc-subunit that confers high-affinity binding and signal transduction (Greenfeder *et al*., 2001).

Both lymphocytes and Eosinophils are derived from a CD34+ hematopoietic progenitor cell in the bone marrow, and mast cells produce IL-5 and GM-CSF, which promote eosinophil growth, maturation, and differentiation (Simon *et al*., 2004). It has been well established that IL-5 is one of the most important cytokines for generation of eosinophils **(**Rothenberg and Hogan, 2006). Confirmed studies have demonstrated that distribution of IL-5 genotype is significantly different among AD patients with eosinophilia, as a result, the IL-5 gene may play a role in AD-related blood eosinophilia(Yamamoto *et al*., 2003).

Human thymic stromal lymphopoietin (TSLP) potently activated CD11c(+) dendritic cells DCs and induced production of the Th2-attracting chemokines TARC (thymus and activation-regulated chemokine; also known as CCL17) and MDC (macrophage-derived chemokine; CCL22), TSLP-activated DCs primed naïve T(H) cells to produce the proallergic cytokines ,IL-5, IL-13 and tumor necrosis factor-alpha, while down-regulating IL-10 and interferon-gamma (Soumelis *et al*., 2002).Th2 cytokine such IL-5 along with other cytokines elevated levels were detected in the skin of AD patients, and levels correlated with IgE levels (Brandt and Sivaprasad,2011).

1.2.7.4 Role of Interleukin 13

Interleukin 13 has been implicated in the pathogenesis of allergic diseases, including AD and asthma, a direct role of IL-13 in AD has not been established IL-13 was produced exclusively in the skin and caused a chronic inflammatory phenotype characterized by xerosis and pruritic eczematous lesions (Zheng *et al*.,

2009). AD which is characterized by a disturbed skin barrier, robust type 2 helper T-cell (Th2)–mediated immune responses to numerous environmental antigens, susceptibility to cutaneous infections and intractable pruritus, is a common chronic skin condition with a worldwide prevalence ,the Th2 cytokines IL-13 are believed to play roles in the pathogenesis of AD (Beck *et al*., 2014). The skin microbiome is changing, and the epidermal barrier function is deteriorating. When IL-13 is overexpressed locally, it has a significant impact on skin biology, including inflammatory cell recruitment (Bieber, 2020) .

Macrophages play a central role in the balance and efficiency of the immune response and are at the interface between innate and adaptive immunity, their phenotype is a delicate equilibrium between the M1 (classical, pro-Th1) and M2 (alternative, pro-Th2) profiles, this balance is regulated by cytokines such as IL-13, a typical pro-M2-Th2 cytokine that has been related to allergic disease and asthma (Martinez Nunez *et al*., 2011).

IL-13 is a cytokine produced by T-cells that has several functions, including inducing the production of IgE, a number of studies have found that cytokines produced locally in the skin play an important role in AD (Obara *et al*., 2002). IL-13 is a cytokine produced primarily by activated Th2 cells (Halim *et al*., 2014). Furthermore IL-13 is known to be involved in various mechanisms of atopic conditions shares promotes B-cell differentiation and is capable of inducing isotope-switching in B cells to produce IgG4 and IgE), it also inhibits inflammatory cytokine production from monocytes and promotes the differentiation and survival of eosinophil and mast cells (Hummelshoj *et al*., 2003).

1.2.7.5 Role of Immunoglobulin E

Hypersensitivity type I is called Atopic anaphylaxis and high fever, which occur immediately through minutes ,the main cause is due to the presence of IgE antibodies (Zina ,2021). Atopic dermatitis is an eczematous, pruritic skin disease that affects 2–20 percent of the general population and has a significant impact on the patient's quality of life, it's often linked to allergic diseases of type I, such as food allergies, asthma, and allergic rhinitis (Thomsen, 2014). The pathophysiology of AD disease is a complicated, involving interactions between genetic, immunological, and environmental factors, skin barrier dysfunction caused by decreased filaggrin expression, as well as a biphasic T-cell-mediated immune response characterized by a Th2 phenotype in childhood and an acute disease phase that shifts to a Th1 signal in the chronic stage, are thought to play key roles in the disease process The elevated level of total serum IgE in approximately 80% of AD patients is a significant symptom of the disease (Kasperkiewicz *et al*., 2018).

The majority of AD patients are atopic, with sensitivities to a variety of allergens, However, since this trait isn't needed for diagnosis, someone can meet the AD requirements without being atopic or allergic to allergens of some kind because of understanding to IgE function in triggering main effector cell types involved in allergic inflammation and its contribution to some other allergic diseases in which patients are sensitized to allergens and have elevated IgE levels, expect to play a role in some other allergic diseases in which patients are sensitized to allergens and have elevated IgE levels (Liu *et al*., 2011). One of IgE most well-known functions is to mediate mast cell activation, results which in the release of

preformed mediators like histamine, as well as cytokines like IL-5 and IL-13, which promote Th2-polarized T cell responses, this shown in Figure (1-3).

IgE may play a role in the development of AD disease by mediating allergen-induced activation of mast cells in the skin, dendritic cells are another type of skin cell that has IgE receptors and can play a role in the immune response mediated by IgE, inflammatory dendritic epidermal cells and Langerhans cells. In those cells, type I IgE receptors (FcRI) are expressed, and IgE increases their expression. The levels of these receptors in both cell types have been found to be higher in people with AD disease (Novak *et al*., 2004).

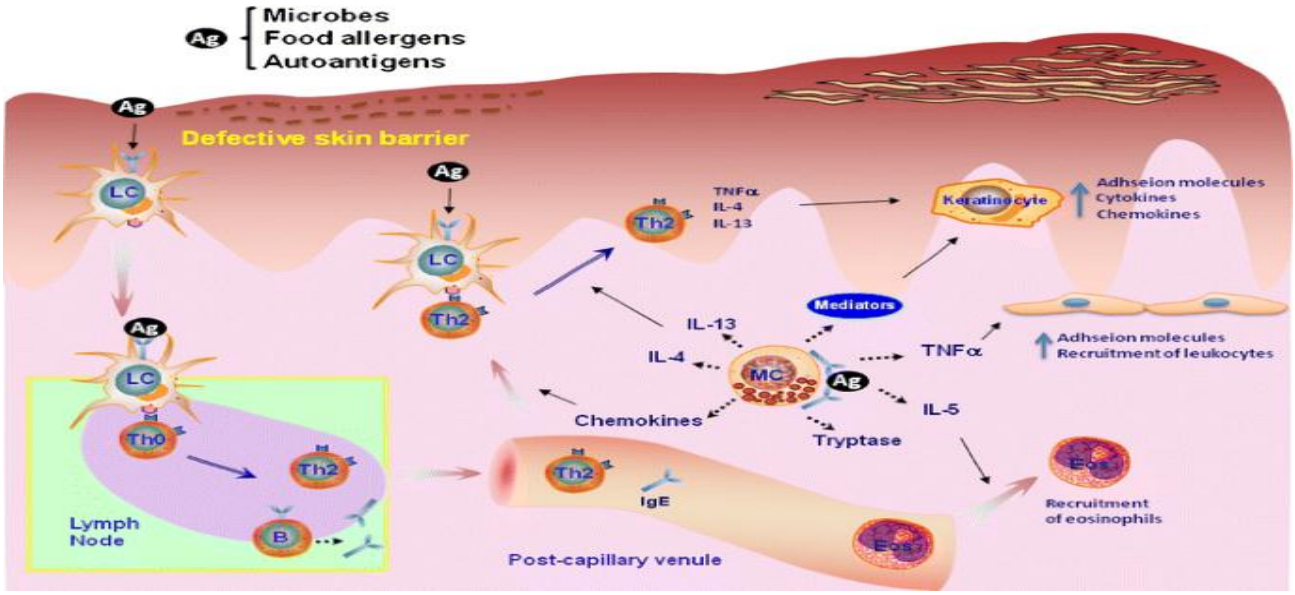


Figure (1-3): Role of IgE in AD (Novak *et al* ., 2004)

Allergens from microorganisms and food can pass through the skin's weak barrier, where they are contracted by Langerhans cells (LCs) through antigen-specific IgE that is restricted to the IgE receptor, antigen-laden LCs migrate to lymph nodes, where they help B cells differentiate into IgE-producing plasma cells by inducing the differentiation of naive CD4+ T cells (Th) into Th2 cells, Th2 cells

travel through the bloodstream and reach the allergen-affected region, LCs reactivate them, which encourages the Th2 allergic inflammatory response, as cell-bound IgE is crosslinked, mast cells in the skin become sensitized, releasing inflammatory mediators, IL-4, IL-5, and IL-13 are examples of interleukins, and TNF- are examples of cytokines and chemokines crosslinking occurs as allergens crosslink IgE bound to cells, chemokines produced by activated mast cells can aid Th2 cell migration into the affected area, Mast cells release the cytokines IL-5 and IL-13, which contribute to the Th2 response up regulation of adhesion molecules on keratinocytes and secretion of cytokines and chemokines by these cells may be induced by mediators such as histamine, chemokines and IL-5 may cause eosinophil infiltration across venules, Autoantigens may play a similar role in the allergic inflammatory response in AD disease (Liu *et al* ., 2011).

1.2.7.6.The Role of Eosinophils

Eosinophils are immune system cells known best for their function in parasite protection, but they also serve as mediators of allergy and asthma, alongside basophils and mast cells AD which illustrate in Figure (1- 4) (Simon *et al*., 2004). They make up anywhere from 1% to 6% of all white blood cells, if not stimulated, they will remain in the bloodstream for 8–12 hours and in tissue for 8–12 days (Young , 2006)**.**

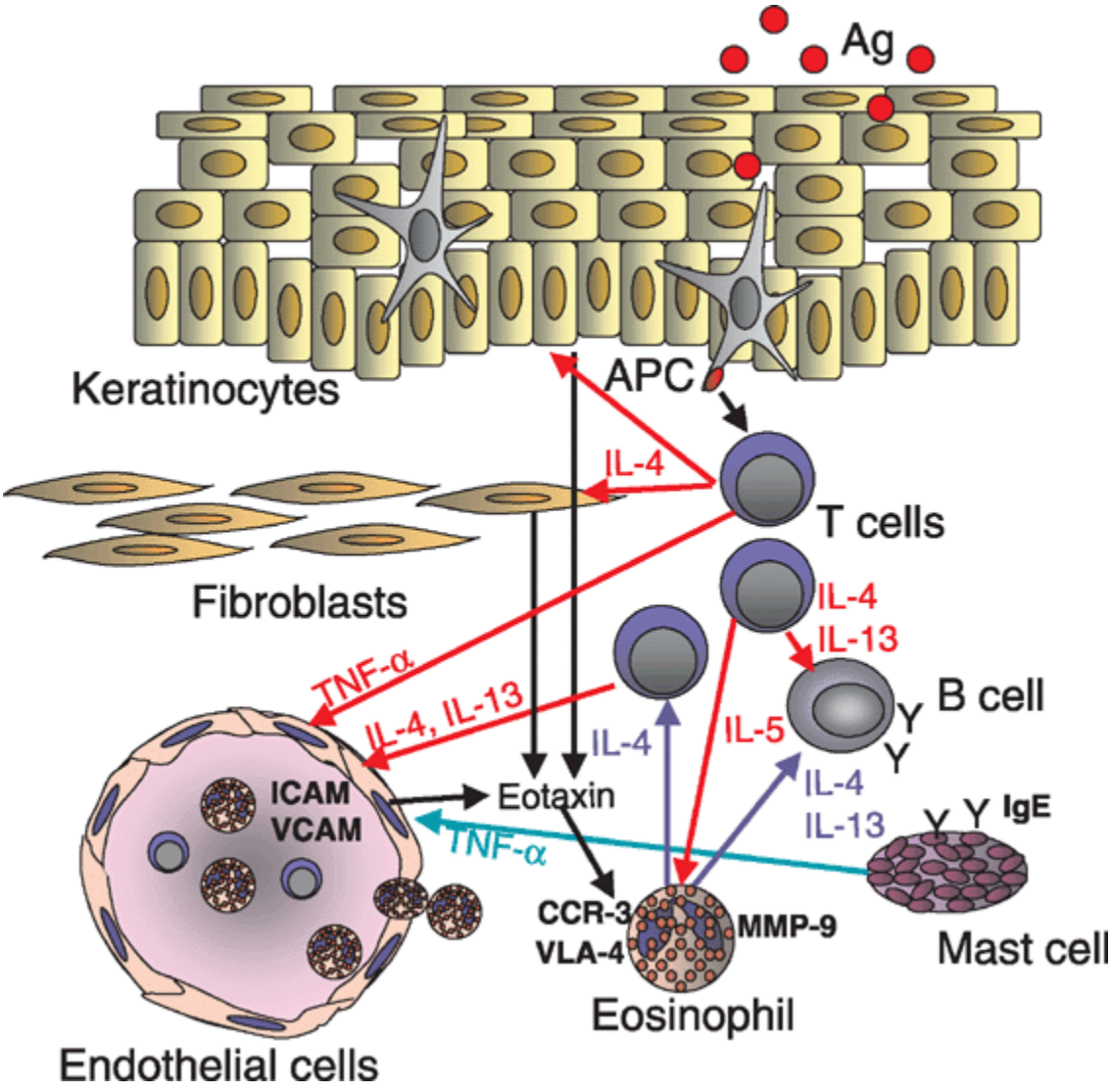


Figure (1-4):The Role of Eosinophils in (Simon *et al.*,2004)

The development of eosinophils and their migration to inflammatory sites are the local activation and distinctive characteristics of AD, Th2 cell activation triggers the development of Th2 cytokines, which assist in the production and function of eosinophils IL-5 and GM-CSF promote the development of eosinophils, In the bone marrow, maturation and differentiation occur, activated eosinophils play a significant immunoregulatory function IL-5 and IL-13 are two cytokines and

chemokines that are secreted by the immune system. In acute AD lesions, there is a change from a Th2-like immune response to a Th1-like immune response in chronic AD lesions occurs when Th2 cytokines activate eosinophils to produce IL-13, The repair and remodeling processes that occur as a result of allergic inflammation are helped by IL-13, they propose that eosinophils play another role in allergic inflammation tissue repair and fibrotic processes, In addition to their proinflammatory function, eosinophils are thought to be involved in tissue repair and fibrotic processes in allergic inflammation, They may play a role by promoting collagen synthesis by way the action of their secreted transforming growth factors (Phipps *et al* ., 2002).

1.8 Skin Bacterial infection in AD

In keratinocytes with AD, increased expression of proinflammatory genes and activation of innate immune responses are linked to chronic skin inflammation (Rebane *et al*., 2014). The skin stratum corneum is highly susceptible to bacterial colonization in patients with AD, and the skin defense system against bacterial invasion appears to be severely disrupted in AD skin, as shown in Figure (1-5) (Arikawa *et al*., 2002).Increased *Corynebacterium bovis* colonization in AD patients resulted in a strong Th2 response in the skin, which is a key feature of acute AD, this could be one way that dysbiosis of the skin microbiome can worsen severity by changing the immune response compared to that seen in AD (Kobayash *et al*., 2015).

During disease flares, the percentage of *Staphylococcus* sequences was higher than at baseline, and this was linked to increased

disease severity, representation of the skin commensal *S. epidermidis* also significantly increased during flares. Increases in *Streptococcus*, *Propionibacterium*, and *Corynebacterium* species were observed following therapy, these findings reveal linkages between microbial communities and inflammatory diseases such as AD, and demonstrate that as compared with culture-based studies, higher resolution examination of microbiota associated with human disease provides novel insights into global shifts of bacteria relevant to disease progression and treatment (Kong *et al*., 2012).

S. aureus while it colonization on the skin of people with AD disease has been linked to disease severity, other members of the skin bacterial community may also play a role, the role of the skin microbiome in preserving normal skin immune function, as well as the negative effects of microbial dysbiosis in driving inflammation, is a promising path for new treatment growth (Williams and Gallo, 2015).

Colonization by *S. aureus* is both a cause and a result of allergic skin inflammation, Skin barrier dysfunction, increased synthesis of *S. aureus* extracellular matrix adhesins, and defective innate immune responses due to reduced development of endogenous antimicrobial peptides are some of the mechanisms by which allergic skin inflammation promotes *S. aureus* colonization, on the other hand, *S. aureus* develops superantigens in the form of exotoxins, through the stimulation of massive T-cells, the position of allergens, and direct stimulation of antigen-presenting cells and keratinocytes, Staphylococcal superantigens (SsAgs) can penetrate the skin barrier and contribute to the persistence and exacerbation of allergic skin inflammation in AD disease, the

expansion of skin-homing cutaneous lymphocyte-associated antigen-positive T-cell, and the augmentation of allergen-induced skin inflammation (Lin *et al*., 2007).

Increased and high abundance of *S. aureus* species in affected skin induces pathophysiology of AD disease, and immune dysfunction has been linked to it, including T helper 2 cells, lymphocyte skewing, reduced AMPs, intensified allergic reactions, and disruption of the skin barrier, when mice are colonized with *S. aureus* in the lab, they develop AD-like lesions, other bacterial species present on normal skin, on the other hand, tend to help in normal immune homeostasis, in comparison to *S. aureus* exacerbation of disease and inflammation, for example, *S. epidermidis*, a common bacterial species found on healthy human skin, can reduce inflammation after an injury and promote development (Nakatsuji *et al*., 2017).

A compromised skin barrier facilitates skin colonization by microbes such as *S. aureus* in adult patients with AD disease, microbial colonization may make it easier for microbial antigens to penetrate the skin, resulting in IgE sensitization, these findings highlight the significance of skin associated microbial colonization and sensitization to microbial allergens in the pathogenesis of eczema (Jinnestal *et al*., 2014). *S. aureus* bacteria play a key role in the pathogenesis of AD disease by secreting toxins, antigens, and proteases that interact with keratinocytes and other inflammatory cells, causing skin hemostasis to be disrupted and the epidermal barrier to be compromised (Maintz and Novak, 2011).

Several *S. aureus* products have been shown to cause pro-inflammatory responses in keratinocytes, furthermore, staphylococcal superantigens may stimulate T-lymphocytes directly via the T-cell receptor (Taskapan and Kumar, 2000). It has also been reported that *S. aureus* produces proteases that cause skin

barrier dysfunction (Hirasawa *et al*., 2009). And that *S. aureus* colonization is linked to skin barrier impairments in AD disease (Jinnestal *et al*., 2014).

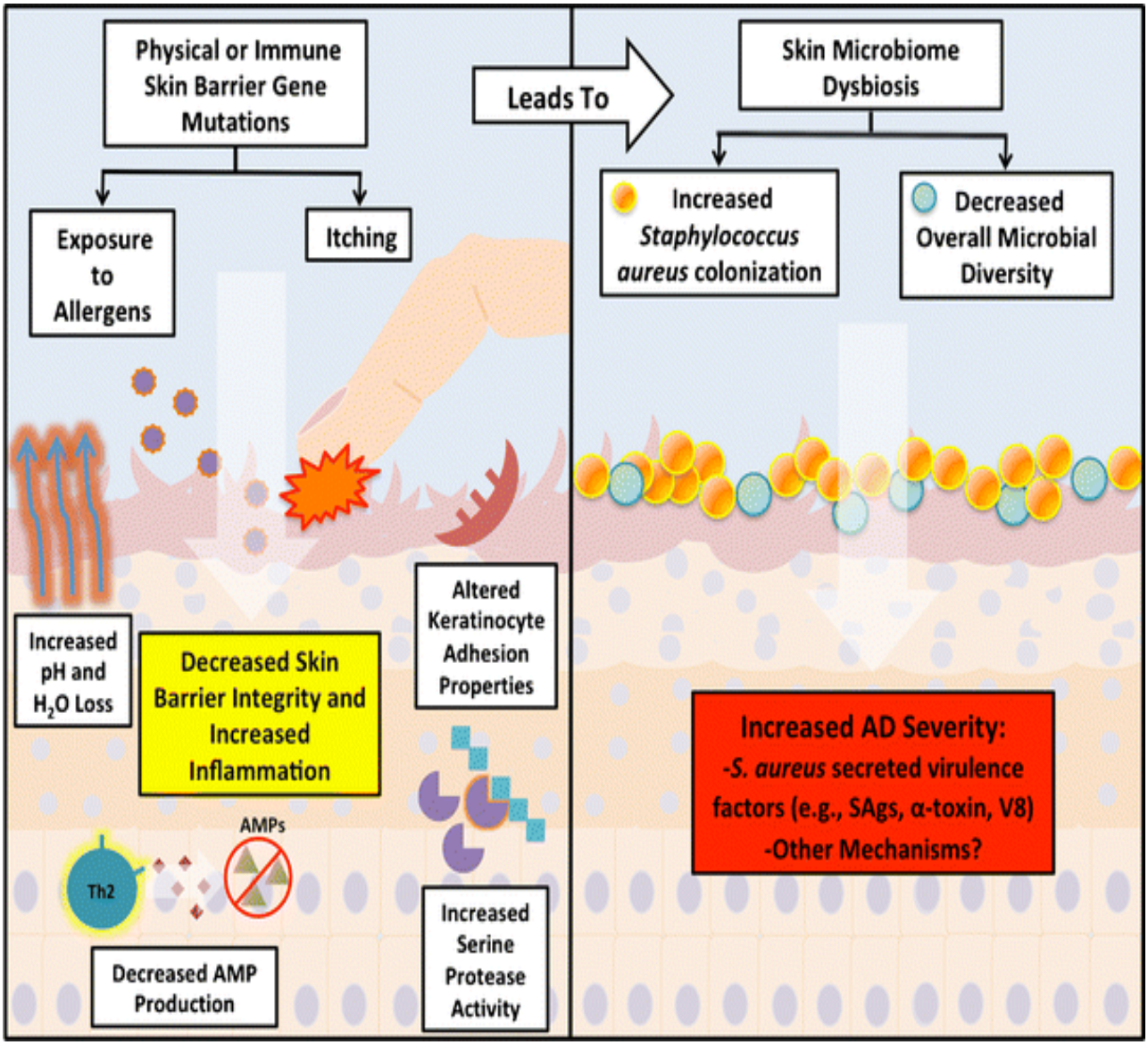


Figure (1-5): Dysbiosis of the skin microbiome in AD patients (Williams and Gallo, 2015).

Chapter Tow
Materials and Methods

2: Materials and Methods

2.1 Materials

2.1.1 Equipments and Tools used

The listed equipments and tools which were used in the present study are illustrated in Table (2-1).

Table (2-1): The equipments and tools and their manufacturers companies

Equipment and tools name	Manufacturers companies
Autoclave	Korea / Kyonggi
Benson burner	Membrane/ Germany
Centrifuge	Hettich/ Germany
Compact system Vitek 2	Swelab / USA
Complete blood cell counter	Swelab / USA
Disposable syringe 5ml	LTD JIANGSU/ China
EDTA tubes	Citotest / China
Electrophoresis unit Gel	BIO-RAD/ England
ELISA reader	Biotech/ USA
Eppendorf tubes 0.5 ml	Merk /Germany
Fume Hood	FASTER Bio 4S/ Italy
Hot plate with magnetic stirrer	Heidoloph / Germany
Incubator	Memmert/ Germany
Jel tubes	P.R./ China
Light microscope	Olympus/ Japan
Micropipettes 5-50 µl ,100-1000 µl, 0.5 µl	Eppendrof /Germany
Nitrile powder free gloves	Bio Basic /Malaysia

Oven	Biobase / China
PCR tubes 50µl, DNA tubes 100 µl	Eppendorf / Oxford
Petri dishes	Biozek/Netherland
Plastic test tubes 10ml	Afco/Jordan
Refrigerator	Arcelik/ turkey
Sensitive electronic balance	Sartorius/ Germany
Sequencers equipment	Macrogen/ korea
Thermocycler- PCR	Biobase / China
Transilluminater UV	Biobase / China
Transport medium Swabs	Citotest/China
UPS	Intex/India
Vortex mixer	Gemmy/Twain

Table (2-2) : Biological and chemical materials and dyes with their manufacturers companies

Biological and chemical materials and dyes	**Manufacturers companies**
Agarose	Wizpio / Korea
Ethanol (70%)	Joud /Iraq
Ethedium promide	Bio basic/ Canada
Glycerol Hydrogen peroxide (3%)	GCC /England
Gram stain kit	Sigma/ Germany
N,N,N,N-Tetra methyl-P- Phenylene Diamine Dihydrochloride	Himedia /India
Plasma-coagulase EDTA (Rabbit	Mast / USA

plasma)	
TBE Buffer	Wizpio / Korea
Methanol	PamReeac/ Spain
Nuclease free water	BioLabs lnc.new /England U.S.A
Sodium Chloride (NaCl)	Fisher /Germany

Table (2-3) : Kits used in the present study

Kits	**manufacturers companies**
DNA extraction kit	iNtRON Biotechnology/ Korea
DNA ladder 100bp	iNtRON Biotechnology/ Korea
IgE ELISA Kit	Demeditec / Germany
IL-13 ELISA Kit	Biosearch Laboratory /China
IL-5 ELISA Kit	Biosearch Laboratory/ China
VITEK 2	Biomerieux/ France

Table (2-4) Culture media and their manufacturers

Media	**Manufacturers companies**
Brain heart infusion broth	HI-Media /India
MacConkey agar, Blood agar base, Nutrient Agar,	TITAN/BIOTECH-India
Mnnitol salt agar	Himedia Accumax/ India

Table (2-5) DNA amplification materials

DNA amplification materials and Contents		
1. DNA Extraction Kit (G-spinTM Genomic DNA) / Intron, Korea		
G-buffer	Pre buffer	Washing buffer A
Washing buffer B	Binding buffer	Elution buffer
Lysozyme powder	Ribonuclease A powder	Proteinase K powder
2-PCR Taq Master Mix with dye /iNtRON Biotechnology/ Korea		
Taq-DNA polymerase	MgCl2	PCR reaction buffer
d (NTP)	Blue dye with stabilizer	
3-100bp Plus Opti-DNA Marker / iNtRON Biotechnology/ Korea		
Marker DNA leader consists of 12 DNA fragment (double-stranded) with a size of 100 bp-1000 bp, 1500 bp, and 3000 bp, it was used to determine the size of double-stranded DNA product from 100bp to 3000bp.		

Table (2-6)The primers Sequences were optioned from Sources

Name of primer	Sequence of primer 5'_3'		Product size (bp)	Source	Origin
27F	F	5'AGA GTT TGA TCC TGG CTC -3'	1400	Dekio *et al* .,2005	Macrogen Korea
1492R	R	5'GGT TAC CTT GTT ACG ACT T-3'	1400		

2.2 Methods

2.2.1 Sterilization

Sterilization of the culture media and solutions used in the study were achieved by using autoclave at temperature 121°C of 15 pound per square Inch for 15 min after adjusted pH for 7.2 . On the other hand , the glasses were sterilized with an electric oven in 180° C for two hours**.**

2.2.2 Preparation of Culture Media

2.2.2.1 Media Used for Bacterial isolation and Identification

All media used in the present study listed in table (2-4) were prepared according to the manufactures instruction steady on their contains. All the media were sterilized autoclave . After sterilization and cooling at 45°C , only blood agar base was completed with 5% of human blood and about 20ml of sterilized media , poured in to sterilize petri dish .Finally, each prepared media labeled and stored at 4°C in refrigerator.

2.2.2.2 Preparation of Maintenance Medium

A. Short Time Storage Medium

It was storage method to maintain the pure culture for one month by preparing the nutrient agar slant in screw-capped tubes and streaked by a charged loop of single colony for bacterial isolate and incubated for 24h in autoclave. Then rolled up with parafilm, held at 4°C (Vandepitte *et al*., 2003).

B. Long Time Storage Medium

This medium was represented by 85ml brain heart infusion broth as a basal medium and supplemented with 15% (v/v) glycerol. It was distributed into 5ml tubes, autoclaved, cooled then saved at 4°C until used. The usage of this medium was to maintain the isolated bacteria at -20 °C for more than 6 months (Vandepitte *et al*., 2003).

2.2.3 Biochemical reagents

2.2.3.1 Normal saline:

It was prepared by dissolving 0.85 g of sodium chloride (NaCl) in 90 ml distilled water ,then complete the volume to 100 ml after that adjust the pH to 7 .Solution was autoclaved and kept in refrigerator until used (Baron *et al*.,1994).

2.2.3.2 Catalase reagent:

It was prepared as concentration of 3 % of hydrogen peroxide(H_2O_2) (3ml H_2O_2 in 90ml of distilled water then completed to 100ml). Reagent was used for detection the ability of bacterial isolates in production of catalase enzyme (Baron *et al*.,1994).

2.2.3.3 Oxidase reagent:

Freshly prepared from adding 0.1 g of N,N,N,N-Tetra methyl-P- Phenylene Diamine Dihydrochloride in 9 ml of distilled water , then the volume was completed to 10 ml in clean , dark and sterile container (Baron *et al*.,1994).

2.2.3. 4 Coagulase Reagent:

The tube coagulase test was conducted following the procedure reported by Kateete *et al*.,(2010). Briefly, using a class tube, one drop of EDTA rabbit plasma was added and proper amount of fresh bacterial suspension, and mixed well by a wooden stick. Up on it, the presence of bound coagulase proteins (clumping factor) on the bacterial cells will lead to clot formation .

2.2.4. Collection of samples:

The case control study was conducted between December 2019 to July 2020 in the laboratory of Biology Department, College of Science , University of Kerbala. The blood samples and skin swabs were collected from 50 patients (30 females and 20 males) with atopic dermatitis , aged from 5 to 35 years , attending at Al- Hussein teaching hospital and some of private laboratory in Karbala Province, Iraq.

Blood samples and skin swabs were also collected from 30 normal individuals (15 males and 15 females) with age range between 5-35 years, who were free from any infection depending on the physician identification and some clinical examination , according to (Sun *et al*., 2019).

A. Blood samples

Five ml of venous blood sample was collected from 50 patients and 30 control group in this study, each blood sample was divided in to two parts. The first one, (2 ml) was placed in EDTA tubes to conduct complete blood count (CBC) .Further the second part (3ml) was placed in the jell tubes and kept to clot at room temperature (25_30) °C. Tubes were then centrifuged at 3000 rpm for 10 min in order to separate serum which was carefully transferred to Eppendorf tubes and preserved at -20°C until used.

B. Swab samples

One hundred skin swabs were taken from area of infection and surrounding area of each the patients and thirty from control group as shown in figure(2-1), by using transport medium swabs for preservation from dryness until transport to laboratory. Samples then were cultured in different type of media after incubation period 24h of 37°C to isolate and determine the more frequent pathogenic bacteria (Sun *et al*., 2019).

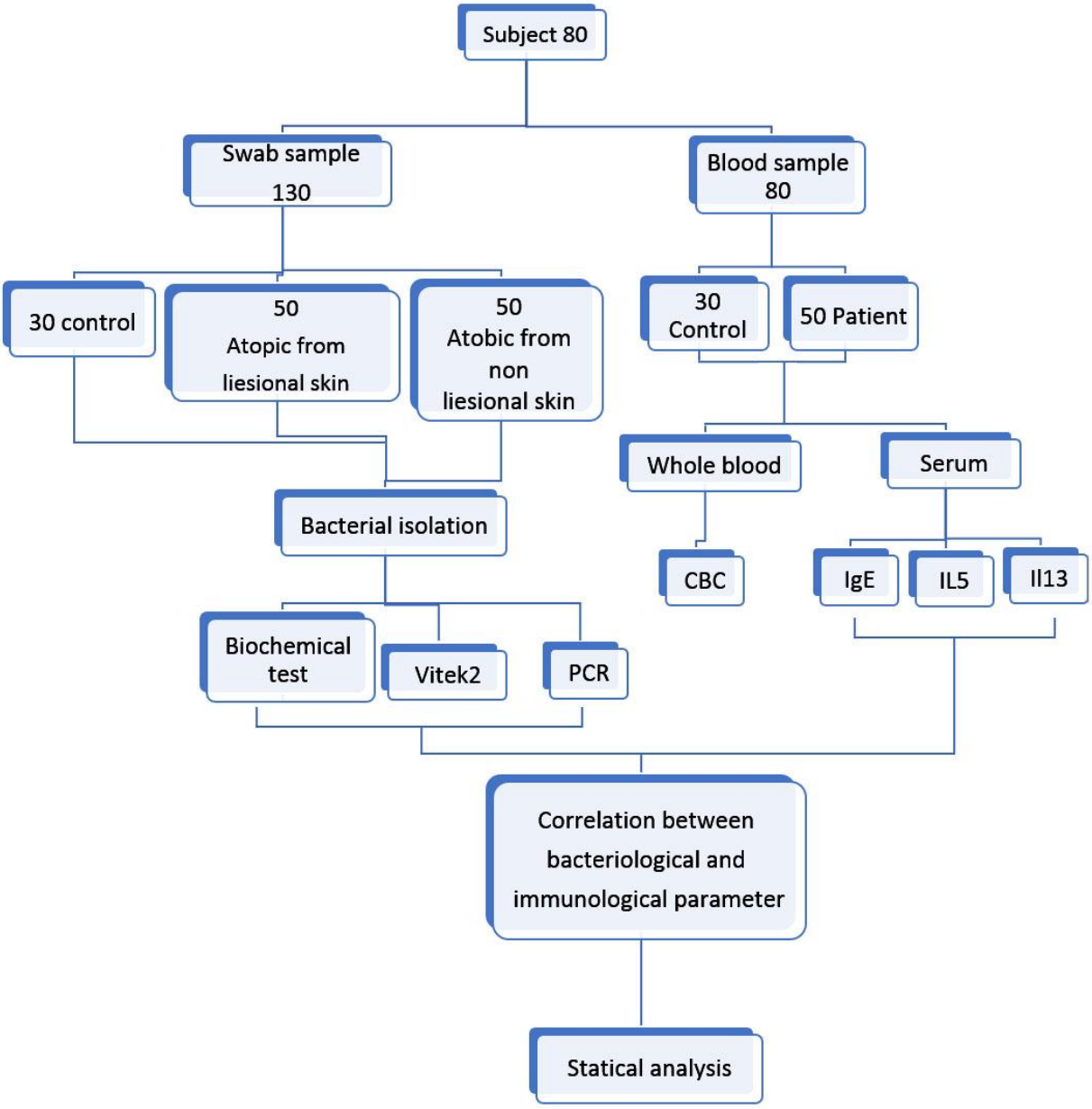


Figure (2-1) Study design

2.2.5 Identification of Isolated Bacteria

Isolated bacteria were diagnosed depending on Atlas *et al*., (1995) Collee *et al*.,(1996) and Goldman and Green (2015).

2.2.5.1. Morphological and Culture Characteristic

The selected bacteria were initially identified according to the colony characteristics such as the, shape, size , color characteristic of the colonies on general and selective media (Goldman and Green,2015) .

2.2.5.2. Biochemical Tests

It was done according to Collee *et al*., (1996) as the flowing steps:

1.Catalase test

This test was performed by transferring part of the bacterial culture to a clean and dry glass slide using a carrier loop ring then a drop of hydrogen peroxide (H_2O_2) 3% was added. The appearance of air bubbles on the surface of the glass slide indicate of the positive result . This test is used to detect the ability of bacterial species for the production of catalase enzyme which convert hydrogen peroxide into oxygen and water.

2.Oxidase test

Drops of the oxidase enzyme reagent were prepared ,and placed on a clean and dry filter paper. Then part of the colony was transferred by wooden sticks on the filter paper for 10-15 sec, the appearance of the dark violet color is sign of positive result.

3.Coagulase test (Free Coagulase test)

The bacteria grew on Nutrient broth and incubated in 37 degree for 18-24 hours, then transfer 0.1ml of bacterial culture to sterile glass tube containing o.5ml from rabbit plasma and tubes were incubated in 37 ° C for 4hours and check the tube every hour because it may degrade again when formed, and negative tube should be left at room temperature for the overnight to notice the thrombus – producing bacteria slowly, plasma clotting is a sign of plasma clotting enzyme.

4.Hemolysin production

The ability of isolated bacteria to analysis of erythrocyte was tested by culture bacteria on blood agar plates which prepared previously as mentioned in section (2.2.3) and incubated at 37° C for 24 hours in aerobically conditions.

2.2.5.3. Vitek Diagnosis System

This system was used for biochemical diagnosis of bacterial species to determine the type of isolated bacteria through the color detection cards GP color –coded diagnostic. Cards were used to diagnose the Gram positive bacteria and the card marked with GN to diagnose bacterial samples , according to the following steps:

1. Samples were cultivated on culture media (nutrient agar) for bacterial growth in the laboratory and then placed in the incubator for 24 hours at 37°C .

2.Then bacterial suspension (pure culture) was prepared by transporting a sufficient number of pure colonies using a loop to a special test tube (made of polystyrene in 12× 75 ml sizes) .This tube contains 3.0 ml of salt solution with adjusting the turbidity according to special measurements by type of colonies ,

3.The test tubes were transferred to the Cassette of the Vitek device and it was transferred to Vitek 2 compact device in the designated area and the machine was turned on.

4.The inoculated cards were passed through a mechanism whereby the transfer tube was cut and the card was pasted before being loaded into the circular incubator. The circular incubator can hold up to 30 or even 60 cards, cards were incubated at 35.5 °C.

2.2.6. The PCR Technique for Identifying Bacteria

2.2.6.1. Bacterial DNA Extraction

In order to extract DNA from pure colonies, the genomic DNA extraction kit was used according to its manufactures company

1. Protocol for Gram_ Positive Bacteria

1. One _two ml of cell were centrifuged at 13,000 rpm for 1 minute to harvest. After that, the supernatant was extracted. Note : after centrifugation, the supernatant was removed and completely suspended by vortex. Do not overload the sample.

2. Fifty µl of Pre-Buffer was added and 3 µl of lysozyme solution, mixed well. Then, incubated at 37c° for a minimum of 15 minutes. Invert the tube every 5 minutes during the incubation to help lyse the cells. After that, 250 µl of G-Buffer solution was applied and thoroughly inverted-mix.

3. Tubes were incubated for 15 minutes at 65°C. Invert the tube every 5 minutes during the incubation to support lysis cells.

4. Two hundred and fifty µl of Binding Buffer was added, and mixed well by using pipet (at least 10 times) or gently vortexing. This step is critical for efficient deproteinization because it allows cell lysates to move through a column more efficiently and increases gDNA binding to column resins.

5 . Cell lysates was loaded on column and centrifuge at 13,000 rpm for 1 min. Note : the maximum volume of the column reservoirs 800 µl. For sample volumes of more than 800 µl, sample load and spin again.

6. To wash, 500µl Washing Buffer A was applied to the column and centrifuged at 13,000 rpm for 1 minute. After that, the solution was extracted. The column was then filled with 500 of Washing Buffer B and centrifuged for 1 minute at 13,000 rpm.

7. Solution was removed and centrifuged for 1 min at 13,000 rpm.

8. The G-spinTM column was placed in a clean 1.5 ml microcentrifuge tube (not provided) and poured 50-200µl . Elution Buffer directly onto the membrane. After that, tube was incubated for 1 minute at room temperature before centrifuging for 1 minute at 13,000 rpm.

2.Tris-Borate EDTA Buffer (TBE Buffer10x) Preparation

This buffer was prepared according to Wizpio-Korea protocol by melting 7.4g of EDTA (disodium salt),61.8g of boric acid and 121.1g of tris to 100ml of RNase-free H2O .The gel running buffer was then made by diluting 100ml to 1liter.It can be kept at room temperature for up to 6 months.

3.Ethidium bromide dye

Ethidium bromide dye made by dissolving (0.01) mg of ethidium bromide powder in 2 ml of D.W, then storing it at 4 °C in a dark container according to (Sambrook and Russel , 2011).

4.Agarose Gel Preparation

The agarose gel was prepared according to the method of Sambrook and Rusell,(2001) by adding agarose to 1x TBE buffer, the concentration of the agarose was different, each based on the loaded sample . The solution was put in the oven for boiling until all of the gel particles was dissolved. The solution was left to 50-60°C prior to combined with 0.5 l/ml ethidium bromide .

2.2. 6.2. Preparation of Primers

Primers were purchased form Humanizing Genomics Macrogen Korea, in the form of a lyophilized product .To achieve a concentration of 100 picomoles per 1µl , primers were dissolved in DNA/RNAase-free water. The final picomoles were calculated by the primer process and stock solution preservation. Each primer was diluted to 10 pmol \ ml and stored in the freezer until required

2.2.6.3 Preparation of PCR Mixture

Polymerase chain reaction (PCR) was prepared according to intron company of Korea. After DNA extracted, PCR was performed for amplifying the 16S rRNA gene to confirmed the identity of bacterial isolates. Each PCR tube mixed contained the following components:

- 12.5 µl of Taq polymerase.
- One µl from each 27F (10 pmol/µl) primer and reversed primer 1492R (10 pmol/µl).

- Three µl of DNA extraction as a template.
- Complete the solution to final volume 25 µl with free nuclease water.

After that, PCR-mix tubes were closed and transferred into the thermocycler. Thermal cycling was conducted in a Gene Amp PCR System 080725 (Multigame, Lab net International Inc.), under the following conditions: initial denaturation at 95°C for 5 min; 30 cycles; denaturation at 95°C for 30 sec; annealing at 65°C for 55sec; elongation at 75°C for 55sec; final extension at 75°C for 5 min.

2.2.6.4. Product Loading and Electrophoresis

Five µl of each PCR product were loaded into agarose gel wells with known well sequences, then DNA ladder was added to one of the wells in the row. After fixing the gel in the electrophoresis chamber, 1X TBE buffer was added to the chamber until it covered the surface of the gel. For 35 minutes, an electric current of 60 volts per centimeter was applied. A Gel Doc system was used to visualize the bands. The DNA ladder was used to measure the product bands. Positive results were identified when the bands were the same size as the target product.

2.2.6.5 Sequence Analysis of Pure Colonies

The PCR products were purified by using a clean kit (Favorv PREP^{TM} PCR Clean–Up Mini (FAVORGEN Biotechnology CORP., KOREA)_according to the manufacturer's instructions.

2.2.6.6.General Protocol

1- 10-100 µl of PCR product was transferred to micro-centrifuge tube, and 5 volumes of FAPC buffer was added and mixed well by vortexing.

2- A FAPC column was inserted into a collection tube and paced.

3-Fill the FAPC column halfway with the sample mixture, centrifuge for 30 seconds at 11,000 rpm, and discard the flow–through.

4- 600 µl of wash buffer (ethanol added) were added to the FAPC column. Then, centrifuge at 11,000 rpm for 30 sec, and then discard the flow through. Then Centrifuge again at full speed (18,000 x g) for an additional 3 minutes to dry the column matrix.

5 - An elution tube was connected to the FAPC column (provided).

6- 40 µl of elusion buffer was added or doubled distilled water (ddH_2O) to the membrane center of the FAPC column .Stand the FAPC column left for 1min.

7- At full speed (18,000 xg) centrifuged or 1min to eluted DNA.

After that, the purified products and primers 27F and 1492R with concentration of 10 pmol were sent for sequencing at Macrogen laboratories (Macrogen, Inc., South Korea). Sequence results were then submitted to a BLAST search in GenBank (http:// blast.ncbi.nlm.nih.gov/Blast.cgi) to compare with the closest known alignment identities for the partial 16S rRNA sequences.

2.2.7 Immunological parameters

2.2.7.1 Total and Differential White Blood Cells Count

The blood samples were collected in EDTA tubes and complete blood count (CBC) indices were directly measured by automated hematological equipment. The analysis was carried out in the following manner:

1. One ml of the blood sample was placed in EDTA tubes and mixed well with the anticoagulant.

2.The tubes were placed in the designated place in the device used to count the white blood cells.

3.The device designed to assess different analysis, including total and differential white blood cells count according to the programming of the device and record the results within one minute and printed.

2.2.7.2. Determine the Level of IgE in the Human Serum

Total IgE level was determined by using the specific kit (ELISA), as the following :_

A. Reagents Preparation

The washing solution: was diluted until using, dilute (1-9%) with distilled water.

- Before using all reagents and samples were bring to room temperature.
- Duplicate standards and samples should be reviewed.
- A standard curve should be established with each assay as shown in Figure (2.2).

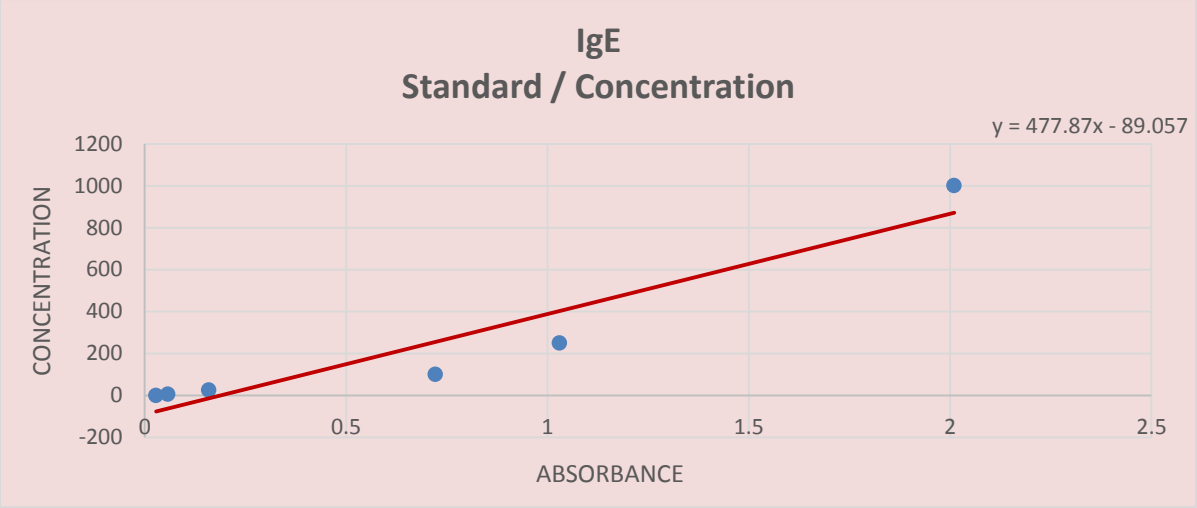


Figure (2.2): Standard Curve of IgE

B. Assay procedure

1. For the standards and samples, as well as a substrate blank, enough microtiter wells were prepared.

2. The undiluted samples were taken up to 10µL each, and the ready-to-use specifications, along with 200 µL of conjugate, were poured into the wells. For the substrate blank, one well was left empty.

3. The plate was covered with the re-usable plate cover and left at room temperature for 30 min.

4. Wells of the plate was empty (dump or aspirate) and 300 µL of diluted washing solution was added. This procedure was repeated totally for three times. Then make of the washing buffer were afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.

5. One hundred µL each of the ready-to-use substrate was taken up and by using pipette put into the wells. This time also the substrate blank was pipetted.

6. Plate was covered with the re-usable plate cover and left at room temperature for 15 min in the dark.

7. To terminate the substrate reaction, 100 µL each of the ready-to-use stop solution was pipetted into the wells. the substrate blank was pipetted as well.

8. After thorough mixing and wiping the bottom of the plate, the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm) was performed. The color is stable for at least 60 min .

2.2.7.3. Determine level of Human Interleukin 5 (IL-5) and IL- 13

Human Il- 5 was measured by using the specific kit (ELISA) according to manufactures' instruction , as the following:

A. Reagents preparation

1.All reagents and micro ELISA plate were brought to room temperature (18-25) before use.

2. The serial dilution for **IL-5 and IL- 13** were prepared from reference standard as show in the Table (2.6).

3.Biotinylated detection Ab was prepared by 1:100 from diluent solution and antibody solution . Biotinylated detection Ab was diluent in the rate 1:99.

4.Concentrated HRP conjugate was prepared by 1:100 from diluent solution and HRP conjugate was diluent in the rate of 1:99.

Table (2.7) Standard serial dilution of IL-5 and IL-13

32pg/ml	Standard No.5	120µl Original Standard + 120µl Standard diluents
16pg/ml	Standard No.4	120µl Standard No.5 + 120µl Standard diluents
8pg/ml	Standard No.3	120µl Standard No.4 + 120µl Standard diluent
4pg/ml	Standard No.2	120µl Standard No.3 + 120µl Standard diluent
2pg/ml	Standard No.1	120µl Standard No.2 + 120µl Standard diluent

stock Standard Solution No.5 No.4 No.3 No.2 No.1

B- Assay procedures

1. Sample injection: a) Blank well: **do not add sample**, - **anti**-IL-5 and Il-13 **antibody labeled with biotin and streptavidin-HRP**; chromogen reagent A & B and stop solution was added, each other step operation was the same.

b) Standard solution well: 50µl standard was added and streptomycin-HRP 50µl (biotin antibodies were united in advance in the standard, so no biotin antibodies were added).

c) Sample well to be tested: 40µl sample was added and then 10µl IL-5 and IL-13 antibodies, and 50µl streptavidin-HRP were added next. Then solution was covered with seal plate membrane and shaken gently to mix. Solution was incubated at 37℃ for 60 min.

2) The washing concentration (30x) with distilled water was diluted for later use as a washing solution.

3) Washing: carefully the seal plate membrane was removed, drain washing solution and shaken off the remainder. Each well was filled with washing solution, stand for 30 sec, then drain. This procedure was repeated for five times then blot the plate.

4) Each well was filled with 50 µl of chromogen reagent A, followed by 50 µl of chromogen reagent B. For color development, the mixture was gently shaken and incubated for 10 min at 37°C away from light.

5) To stop the reaction, 50 µl of stop solution was applied to each well (color changes from blue to yellow immediately at that moment).

6) The absorbance (OD) of each well was measured one by one under 450 nm wavelength, which should be completed within 10 min after the stop solution was applied.

7) The linear regression equation of the standard curve was determined based on standard concentrations and OD values, as shown in Figure (2.3) and |(2-4). The concentration of the related sample was determined based on the OD value of the samples. Statistical software was used as well.

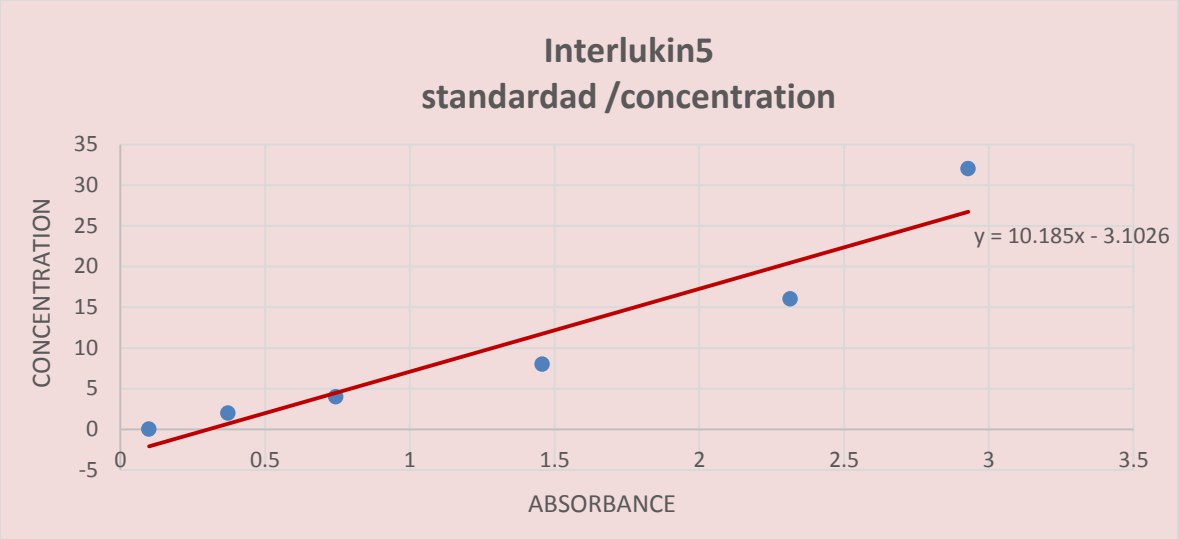
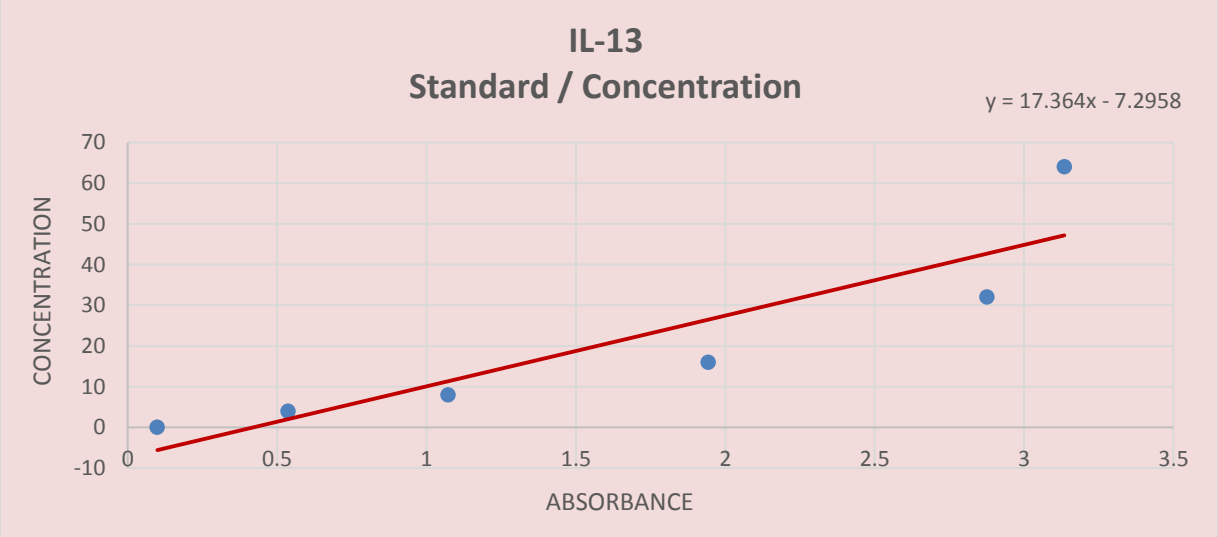


Figure (2.3): Standard Curve of 5 (IL_5)



Figure(2.4): Standard curve of 13 (IL_13).

2.2.8 Statistical Analysis

Using the social sciences statistical software package (MiniTab statistical software version17, IBM (Pennsylvania, USA)), statistical analysis was conducted and the results were expressed as mean±standard deviations (Mean±S.D). Using Student's t-test for two independent means and one way ANOVA for more than two independent means, statistical analysis was conducted on the importance of quantitative data differences (P-values of around <0.05) (Al Ghanmi,2016).

2.2.9 Ethical Statement

The biology department local committee agreed to the experiments mentioned in this thesis and all volunteers give formal consent and details and advantages were given to the patients based on research and copy of the completed medical analysis. The study was undertaken by the University of Kerbala under supervision of doctors in Al Hussein Hospital .

Chapter Three

Results and Discussions

3.Results and Discussion

3.1 Distribution of Atopic Dermatitis Infection According to the Age and Gender

During the current study, swabs and blood samples, were collected from only 50 patients with AD in Kerbala province, (30females and 20 males, their age from 5-35 years) and 30 healthy persons as a control group (15 females and 15 males, aged from 5-35 years).

The results showed that, the percentage of AD infection in 30females (60%) were higher than in 20males (40%) these percentages are presented in Table (3-1). In addition, there were significant differences between patients age groups, the highest percentage infection (60%) was in the patients of 5-15 age category while the lowest infection percentage (18%) was in the 26-35 group, which are shown in Table (3-1).

The present results were in agreement with Ramirez *et al* ., (2019) who demonstrated that, AD was more prevalent between the ages group from 2-16, and children with AD were more likely to be female, who have comorbid asthma or have allergic rhinitis. In addition, the prevalence and persistence of AD were highest in urban children in the United states who were black female and of 5-15 age groups (McKenzie and Silverberg , 2019). Previous studies explain that the AD is one of the most common skin disorders and affects about 5-20% of children with a lower incidence in adulthood in the worldwide (Illi *et al*., 2004 ; carroll *et al*., 2005).

This disease is a common disorder in childhood, but in a large proportion of infants, that the prognosis is often determined by the degree of atopic sensitization in the first decade of life, AD is regarded as one of the most common skin

disorders in young children, and its affects up to 20% of children and up to 3% of adults (Krakowski *et al*., 2008).

Some studies have shown that AD prevalence is still growing, particularly in countries with low incomes and typically it is first to occurs early in the life and frequently precedes other allergic disorders such as asthma (Hong *et al*., 2012 ; Nutten, 2015). There for all major allergic diseases in children are relatively high.

Table (3-1): Distribution of AD according to gender and Age

Patients gender	Frequency	Percentage of AD	Percentage of bacterial infection in AD patients
Male	20	40%	28%
Female	30	60%	44%
Distribution of AD according to age			
Age categories	Frequency	Percentage of AD	Percentage of bacterial infection in AD patients
5-15	30	60%	44%
16-25	11	22%	12%
26-35	9	18%	12%

3.2. Identification of Bacterial Isolates

The bacteria were isolated from only 34 patients after their skin swabs were cultured on a general and selective media, and after 24h of incubation on 37C°. The growing bacteria were identified biochemically according to methods described by Collee *et al* (1996) Goldman and Green (2015). These isolates were identified further by using Vitek2 system as shown in (appendix 9), that is a

new automatic system for the identification and susceptibility testing for the most clinically important bacteria.

After that more common bacterial species were selected (10 isolate) from lesional ,non lesional loci of AD patients and from control group, to confirm their identification by using PCR , as shown in Figure (3-1) .The PCR products were sent for sequencing and the results of sequences were illustrated in Table (3-2) and Appendix (6and 7).

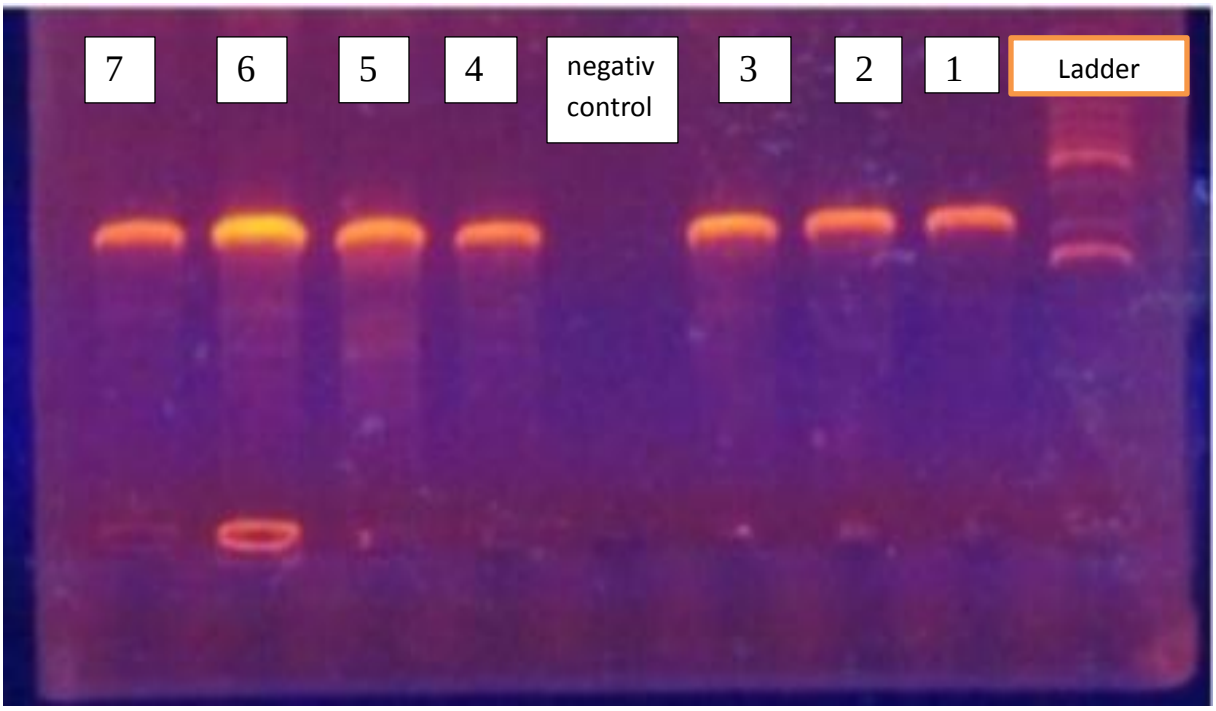


Figure (3.1) : The gel electrophoresis showed that, the patterns for tested bacteria obtained from patients with AD and control groups, Samples codes, 1 (*S. aureus* MK809240), 2 (*S.aureus* KR862289), 3 (*S. haemolyticus* KY271394), 4(*S. aureus*) KY608137, **5 (*S. epidermidis* MT445233), 6 (*S. epidermidis* MT605363) and 7 (*S. epidermidis* MF428819). Product size=** 1400 bp and **ladder=** 100bp

Table (3.2) Sequence results of bacterial isolates

Name of bacterial isolate	Percentage of bacterial isolate	Accession number
Staphylococcus aureus	98.08%	MK809240
Staphylococcus aureus	97.07 %	KR862289
Staphylococcus haemolyticus	94.50%	KY271394
Staphylococcus aureus	97.40%	KY608137
Staphylococcus epidermidis	99.24%	MT445233
Staphylococcus epidermidis	96.02%	MT605363
Staphylococcus epidermidis	99.2%	MF428819
Staphylococcus epidermidis	94.3%	KM013932
Staphylococcus aureus	96.50 %	CP053356
Staphylococcus aureus	93.6%	KX456109
Staphylococcus aureus	97%	AP023034
Bacillus spp	97.9%	LC488930
Bacillus thuringiensis	97.11%	MH921613
Bacillus pumilus	95.5%	HM480380
Bacillus cereus	92.4%	MK675106

Human microbiome has greatly improved with the availability of cost-effective and high-throughput culture-independent techniques, including 16S rRNA gene sequencing and advanced bioinformatics, which has significantly improved our understanding of the human microbiome (Kuczynski *etal* ., 2012). The results of DNA sequencing from isolated bacteria in the AD patients skin is agreed to study of Williams and Gallo, (2015) who stated that, during AD, the culture of bacterial changes in the skin and recent approaches to DNA sequencing have been further identified as *S.aureus* and the it colonization has been studied in

a great detail and seems to be an important factor that exacerbates the disease, other species, however, such as *S. epidermidis* also show increased colonization, and AD pathogenesis can be modulated , Furthermore, similar to Gong *et al*., (2006) who revealed, on infected skin, AD patients have an overall rise in Staphylococcae species colonization and a decline in the number of different types of bacteria (microbial diversity).

Nakatsuji *et al*., (2017) mentioned that, *S. aureus* colonization of the skin increase in AD, while *S. epidermidis* colonization increases to a lesser extent, as previously seen, dysbiosis occurs when the development of these colonies are not followed by a rise in the other bacteria present on normal skin, by use the 16S rRNA gene sequence and skin swabs from healthy non-AD subjects and AD patients who were lesional and non-lesional.

3.2.1 percentage of Bacterial Isolates

Data of microbiology obtained from the present study showed that the Gram positive bacteria were recorded with high percentage than the Gram negative bacteria as studied patients group in the Table (3-3).These positions are represented by AD lesional area from which thirty four (34) patients, bacterial isolation were obtained. The Gram positive bacteria were 31 isolates (91%) represented by *S. aureus* (70.5%) were the commonest isolated genera followed by Bacillus spp (8.8%), *K. rhizophila*(5.9%), *S. epidermidis*(2.9%) , *S. heamolyticus*(2.9%), but only 3 isolates (8.8%) were Gram negative bacteria that represented by *Pseudomonas* spp from lesional loci.

In addition Gram positive bacteria included *S.aureus , S.epidermidis and S.heamolyticus* were isolated from surrounding area of AD injury area in high percentage compared to Gram negative bacteria, one isolates (3%) that represented

by (*Pseudomonas stutzeri*). Furthermore the profile of bacterial isolated from skin of the control group were represented by low percentage of *S.aureus* 4 isolates (20%) with high percentage of normal flora 16 isolates (80%) *S.epidermidis*. These results show that, the percentage of pathogenic bacteria were high in the patients compared to the control group .

Table (3-3) Percentage of bacterial isolates from the patients and control groups

Position of swabs	**Negative culture**	**Positive culture**	**Gram positive**	**Gram negative**
AD lesional area n=50	16	34	*24S.aureus*(70.5%),2 *Kokuria rhizophila* (5.9%), 1*S.heamolyticus*(2.9%) ,1*S.epidermidis* (2.9%) 3*Bacillus* spp (8.8%)	3*Pseudomonas stutzeri* (8.8%)
AD surrounding area n=50	19	31	*20S.aureus*(64%), 6*S.epidermidis(*19%) 4 *S. heamolyticus*(12% *)*	*1Pseudomonas stutzeri* (3%)
Skin swab from normal person n=30	10	20	4*S.aureus*(20%), 16*S.epidermidis* (80%)	–

These results indicated that the variant in immune system of patients effect as predisposition factor for colonization of bacteria on skin of AD patients ;this is in agreement with study of Iraqi researcher Zina,(2021) who noticed that *Staphylococcus* spp , *Bacillus* spp , *Pseudomonas* spp, were the more prevalent

isolated bacterial from lesional loci of AD patients and same it were found in non lesional skin, but in a lower percentage.

Yamazaki *et al*., (2017) explain that *S. aureus* selectively colonize the injured skin of AD patients, although this bacterium is absent from the skin in most healthy individuals, AD is a multifactorial chronic inflammatory disease predominantly caused by a genetic predisposition that contributes to environmental factors being hypersensitive and *S. aureus* colonization being commonly involved (Casas *et al*., 2008).

Both innate and adaptive immune responses can be modulated by microorganisms, the development of the immune system in children, as well as the cutaneous microbiome later in life leads to the growth and course of skin disease like asthma and AD, consequently, a reduction in microbiome diversity in patients with AD correlates with disease severity and increased pathogenic bacteria such as *S.aureus* colonization (Paller *etal*., 2019).

AD is a multifactorial disorder involving, a dysfunctional epidermal barrier triggered by altered keratinocyte differentiation, gene expression and irregular extracellular lipid material, leading to increased permeation of allergens irritants and microbes (Mu and Zhang, 2020).

However, recent research has shown that the microbiota of the skin plays a role in the regulation of skin immune responses as well as the development of inflammatory skin diseases, furthermore, chronic skin inflammation in AD further decreases the skin barrier's function, leading to positive feedback (Egawa and Kabashima , 2016) .Strikingly, skin inflammation occurs independently of adaptive immunity and is associated with IL-5 cutaneous expansion, creating type 2 innate lymphoid cells, the skin innate immune system is a central determinant of the

epidermis physical, chemical, microbial, and immunological barrier functions (Saunders *et al*., 2016).

A malfunction may result in an inadequate host response to a pathogen or a persistent inflammatory state because AD is the most prevalent inflammatory condition (Kuo *et al*., 2013) . *S.aureus* was established by these observations. As a potent inducer of eczematous dermatitis through an immunological pathway that has yet to be determined, *S. aureus* is a huge burden for patients and caregivers (Kobayashi *et al*., 2015). AD is characterized by a hereditary predisposition characterized by an impaired skin barrier and inflammation that is primarily T-helper-2 (Simon *et al*., 2019).

The results of the current study agreed with the study of Geoghegan *etal*., (2018) during flares, *S. aureus* is commonly isolated from the skin of patients with AD.

As well as similar results were reported from another study achieved by (Williams and Gallo, 2015) The presence of *S. aureus* on the skin of people with AD disease has been linked to the severity of the disease, but other organisms in the skin bacterial population can also play a role.

Byrd *et al*., (2017) who used sequencing to analyze the species and strains present at baseline and during flares in pediatric AD, They observed that patients with more mild disease had more *S. epidermidis* detected in flares and that those with severe disease were colonized by dominant clonal *S. aureus* strains.

Mechanisms which promoting skin colonization by *S. aureus* includes complex interactions among several factors, apart from increased *S. aureus*

adhesion in atopic skin, a deficiency in the innate immune response caused by a lack of microorganism growth restriction also leads to susceptibility to *S. aureus* colonization and infection (Nowicka and Grywalska, 2018).

3.3. Immunological Parameters of Patients

3.3.1. Total and Differential White Blood Cells Count

Results presented in Table (3-4) showed a significant increase ($P < 0.05$) in WBCs count in patients with AD in the females (8.48) and males (7.80) compared to males (6.86) and females (7.10) in the control group respectively as shown in (appendix 5).

These in agreement with the current results of Seo *et al*., (2019) that he reported in his research, significant increase in WBCs count in AD patient compared to control group. And in agree with Ying and Wencong, (2017) who stated that , patients with AD had significantly higher WBCs. These test results were found to be incompatible with Zina ,(2021) that reveals most of the AD patients have normal WBCs counting number.

The results illustrated in Table (3-4) showed that significant variant of differential WBCs count, the mean of neutrophils cell showed significant increase in females (51.72) compared to males (46.21) as shown in (Appendix 1).In addition the lymphocytes cell were significant increase in males (37.42) in compared to females (36.02) but is non-significant increase in males compared to control group as shown in (Appendix2).

The eosinophils and basophils show significant increase ($P < 0.05$) in the both sex compared to control group (Appendix 3,4) .These results of increase eosinophils , basophils, lymphocytes, neutrophils are in agreement with Ying and

Wencong,(2017) who determined an increase of differential WBCs count to be obvious in patients with AD.

Nikhil *et al*., (2013) demonstrated an unusual elevated dermal neutrophil infiltrates in AD skin patients. Moreover, Carolyn *et al* ., (2019) showed that, the neutrophils skin-infiltrating are mostly key indications to the itching actions in those patients having AD, the most dominating cases of disorders of the chronic itching.

Table (3-4)Total and differential blood cells count in the study group

	Female		**Male**	
	Patients n=30 Means ± SD	Control n=15 Means ± SD	Patients n=20 Means ± SD	Control n=15 Means ± SD
WBCs	8.48 ± 2.87^{a}	7.10 ± 1.12^{a}	7.80 ± 2.18^{a}	6.86 ± 1.25^{a}
Neutrophils	51.72 ± 12.40^{a}	41.44 ± 4.18^{b}	48.75 ± 9.71^{ab}	46.21 ± 6.15^{ab}
Lymphocytes	36.02 ± 12.24^{a}	35.84 ± 6.08^{a}	37.42 ± 9.22^{a}	40.14 ± 7.48^{a}
Eosinophils	4.37 ± 1.10^{a}	0.80 ± 0.09^{b}	5.01 ± 1.01^{a}	0.89 ± 0.13^{b}
Basophils	0.36 ± 0.21^{a}	0.11 ± 0.03^{b}	0.45 ± 0.2^{a}	0.14 ± 0.05^{b}

In AD patients , there were abnormities in the count of WBCs which led to immune imbalance and grow worse of AD (Chung *et al*., 2005).

The diverse immune network in the AD cutaneous inflammation is made up of adaptive immune cells (B cells and T cells) and innate immune cells (mast cells, eosinophils, basophils, dendritic cells in various types, innate lymphocytes, and myeloid suppressor cells) with the exception of CD4+ T helper cells, which have been shown to drive AD in a variety of experiments, clinical trials, and

mechanistic analyses, few experimental setups and studies attempting to uncover a role for these distinct cell types in AD allow defining one of these cell types as causal for cutaneous inflammation (Staumont *et al.*, 2014).

In AD patients, eosinophilic granulocytes and eosinophilic granulate proteins are deposited in the skin lesions, IL-5 expression is associated with increased Th2 activity in the acute phase of AD and results in increased eosinophilopoiesis, eosinophilic activation, and chemotaxis as well as patients with extreme AD had higher eosinophilia than those with mild to moderate AD, but the difference was not important (Jenerowicz *et al* ., 2007).

Eosinophils are also important participants in the AD skin inflammation ECP (eosinophil cationic protein) serum level, which is considered a marker of eosinophil activation, in addition to IgE, dendritic cells, basophils, and mast cells, confirming the role of eosinophils in the pathogenesis of the disease (Sirufo *et al*., 2018) .

The current results explain clearly that variant of WBCs counts in AD patients may be predisposing factors for exacerbation and emergence of skin bacterial infection in compare to control group where the increase in the percentage of bacterial infection in AD patient was consistence with the increased in WBCs count as the first inflammatory indicators in those patient .

During a bacterial infection, large numbers of neutrophils are consumed, thus, from onset of infection to recovery, dynamic changes occur in WBCs count and left shift data, reflecting the mild to serious condition of the bacterial infection(Honda *et al* .,2016).

The maturation of WBCs in the bone marrow and their release into the circulating pool are affected by colony stimulating factors, interleukins, tumor necrosis factor, and complement components, and an elevated neutrophil count typically represents the normal response of bone marrow to an infectious or inflammatory process, If neutrophils are activated into the circulating pool, they only remain in the blood for a few hours before returning to the bone marrow to replenish the circulating pool with neutrophils (the volume in the circulating pool is replenished four to six times per day in a healthy person), As a result, the WBCs (almost the same as absolute neutrophil) covariates with the balance of neutrophil demand and supply, changes rapidly, After the amount of segmented neutrophils (mature cells) deposited in the bone marrow decreases, band cells and less mature cells emerge in the circulating pool (Ishimine *et al*., 2013).

During the early stages of bacterial infection, neutrophils from the circulating pool migrate easily to the infected site, and those from the marginal pool mobilize in the circulating pool, but neutrophils from the bone marrow are not supplied to the circulating pool. Until the bone marrow supplies a sufficient number of neutrophils, the WBC count falls below the reference range (probably several hours after the onset of bacterial infection) (Ishimine *et al*., 2013).

3.3.2 IL-5 and IL-13 Levels in Studies Groups

The serum levels of IL-5 and IL-13 in patients group have been determined in Figure (3-2) (3-3) . The level of IL-5 and IL-13 were significantly higher in -patients (P <0.05) in level of IL-5 (13.3) and IL-13(23.6) in patients compared to control group (2.2) (2.06) respectively .

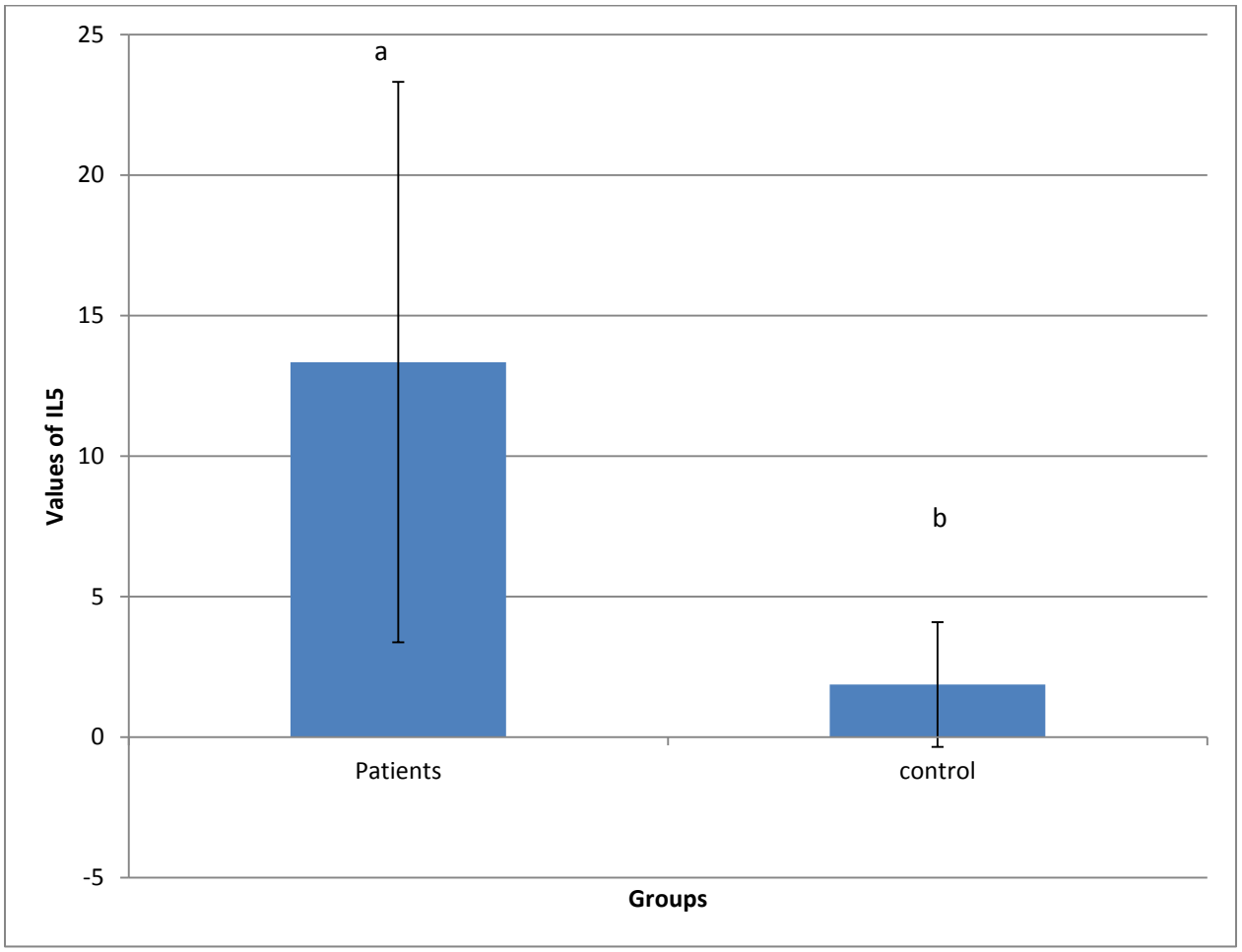


Figure (3-2): Serum level of IL5 in patients and control groups. Data are presented as mean ± SD in each group. Bars with different letters in the same gender are significantly different ($P < 0.05$).

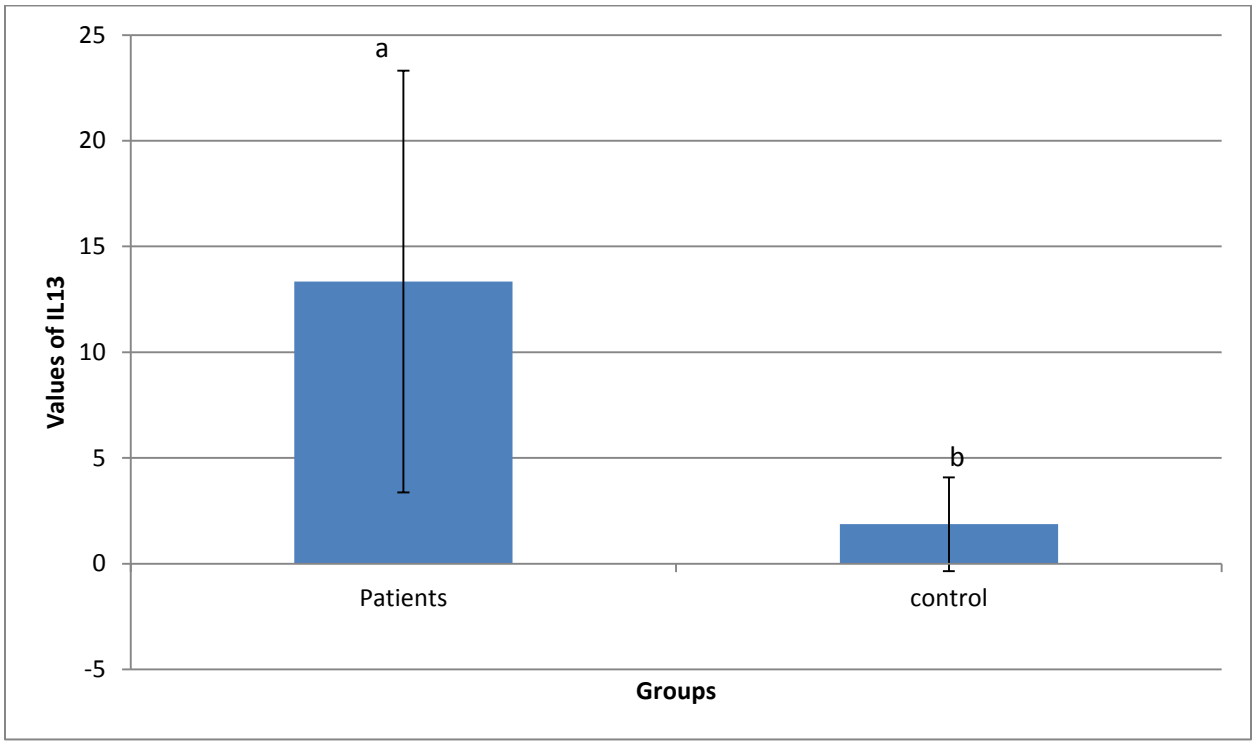


Figure (3-3): Serum level of IL13 in patients and control groups. Data are presented as mean ± SD in each group. Bars with different letters in the same gender are significantly different ($P < 0.05$).

These results are in agreement with Simon *et al* .,(2004), Patients with both extrinsic and intrinsic AD released higher levels of IL-5 than normal controls, which is an essential cytokine for eosinophil production, survival, and proliferation. IL-5 levels were found to be elevated in the skin of AD patients, along with other cytokines, and levels were linked to IgE levels.

The current results support the IL-13 central position in the pathophysiology of AD these results are in agreement with Biber (2020) who explain that the, IL13 is substantially higher in patients with AD disease, making it one of the primary drivers of the disease, The epidermal barrier feature and the local immune response are both influenced by IL-13, As a result, a range of novel strategies are being developed, including those that target this cytokine and its receptors, AntiIL-13 biologics are now in the pivotal stages of their clinical development programs, and

these trials will ideally confirm that targeting IL-13 is a new choice for treating patients with moderate to serious AD, The method of an IL-13 related, biomarker-based stratification of the complex phenotype of AD appears promising and worthy of consideration in future clinical development programs, potentially paving the way for a precision medicine approach to AD management, The interleukin 13 (IL-13) cytokines and inflammatory pathways have been identified as important for the pathophysiology of AD (Hamann and Thyssen ,2018) .

In addition to basophils and mast cells, IL-5 involved in the differentiation, maturation, migration, growth, survival, trafficking and effector role of eosinophils in the blood and local tissue (Molfino *etal*., 2012) .

A dense infiltrate of activated CD4+ T cells can be seen in the dermis in AD disease, particularly in acute lesions, and analysis of atopic patch test lesions has been helpful, cytokine expression analysis showed that early lesion T helper cellsTh2 cells contain the cytokines IL-4, IL-5, and IL-13, as a result, the theory that has been established over the last 20 years is that skin Th2 cytokines stimulate cutaneous inflammation in AD disease, the Th2-induced isotype switch in B cells leading to the development of IgE is frequently cited as examples of Th2-associated pathology and IL-5 promoting eosinophil maturation and survival are highlighted to play a role in certain forms of AD and other atopic diseases (Ortega *etal* .,2014;Guttman *etal* ., 2020,)

3.3.3 IgE Level in Study Groups

Another test in a concern with the AD patients is considered to be very important in many aspects, especially, it can be considered as a predictable indication of AD.

The results in Figure (3-4) show increase in serum level of IgE in patients with AD (378.2) compared to control group (65.1), this results accordance with Sampson *et al*, (2010). The inspection of the IgE levels with age groups reveals that AD children with *S. aureus* infection, generally have higher levels of IgE. Additionlly, Somani (2008) who reached that, in AD disease patients, serum IgE levels are elevated, and the highest levels seen in people with ages between 10 and 20 years. In the pattern of sensitizations, this study also noted age dependence and children.

The imbalance of Th2 to Th1 cytokines found in AD can cause changes in immune responses mediated by cells and promote IgE-mediated hypersensitivity, both of which appear to play a role in the development of AD (Boothe *etal* .,2017). The elevated level of total serum IgE seen in around 80% of AD patients is a major symptom of the disease and IgE antibody responses to various environmental allergens are actually mounting in AD patients (Thomsen, 2014).

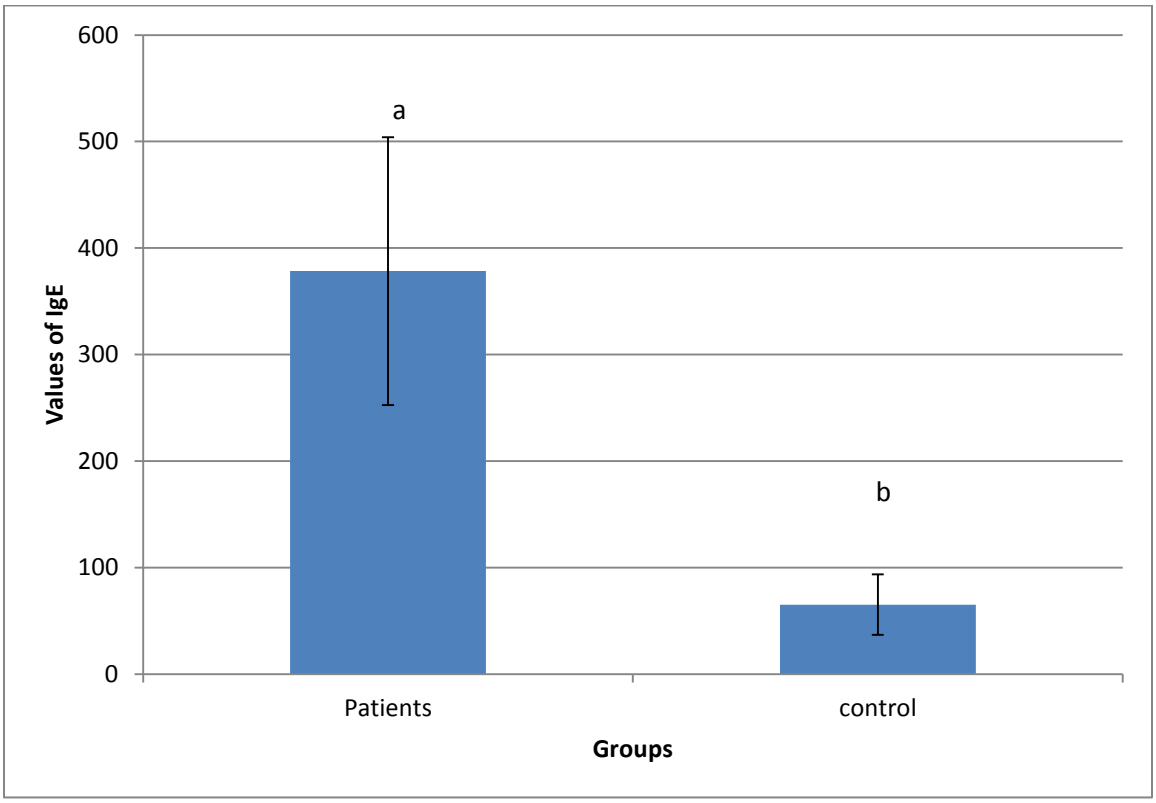


Figure (3-4): Serum level of IgE in patients and control groups. Data are presented as mean ± SD in each group. Bars with different letters in the same gender are significantly different ($P < 0.05$).

The results in Table (3-5) and Appendix (8), show positive relationship between the IgE ,IL-5 and IL-13 serum level with skin bacterial infection in AD patients, As there was a clear increase in the rate of IgE (382.2),IL-5(13.3) and IL-13 (29.6) in patients with AD and local bacterial infection in compare to AD patients without bacterial infection(291.7), (8.2), (13.8) as well as control group (65.1),(1.8),(2.0) respectively.

Table (3-5) The relationship between level of IL13 ,IL5 and IgE in Bacterial , non-Bacterial AD patients and control group.

	IgE	IL-5	IL-13
Bacterial Infected group n=34 Means ± SD	**382.2±129.4**	**13.3±9.7**	**29.6±16.1**
Non Bacterial Infected group n=16 Means ± SD	**291.7±143.1**	**8.2±7.1**	**13.8±8.14**
Control group n=30 Means ± SD	**65.1±28.4**	**1.8±2.2**	**2.1±1.2**

These results are in agreement with researchers Sonkoly *et al*., (2006) suggest that infection with *S. aureus* predisposes people to produce Th2 responses. It's still unclear if faulty immune responses predispose patients to *S. aureus* infections, which cause Th2 responses and AD disease .

The researchers Brandt and Sivaprasad, (2011) would suggest that, the attenuated innate immune responses result in persistent of *S. aureus* colonization ,epicutaneous delivery of the *S. aureus* exotoxin SEB, on the other hand, is enough to cause a Th2 response, increased dermal eosinophilia, and immunoglobulins like IgE in mice.

Increased skin pH, decreased antimicrobial peptides, and Th2 cytokines like IL-5 and IL-13 may all play a pivotal role in the increased risk of skin infections associated with AD disease, bacterial virulence, such as that of methicillin-resistant *S. aureus* (MRSA*)*, is also a factor to increases the amount of superantigens produced, lead to increasing their ability to cause infection and more severe cutaneous inflammation, according to new studies, the skin microbiome, which

includes *S. epidermidis* and other coagulase-negative staphylococci, may help people with AD disease escape *S. aureus* skin infections (Ong and Leung, 2016).

As a result of all the above mentioned conclusions of the present study in addition to the remarks of other researchers in concern with the mentioned findings, It is necessary to highlight the need of continual skin care in keeping the symptoms of AD patients from worsening by keeping the skin barrier from being compromised or damaged as a result of the pathology of AD, which is a common indicator of filaggrin (a protein found in the skin cells) , abnormal growth of gram-positive bacteria and the increase of T helper cell type 2 (Th2) cytokines as (Kozo *et al*. 2019) mentioned .

Finally, It is safe to advise AD patients to follow a routine of frequent skin washing while avoiding dryness to help strengthen the barrier's activities, hence suppressing bacterial development issues and achieving the ideal Th1/Th2 ratio balance as proposed by many other researchers including Peng and Novak, (2015). The use of soap and detergents raises the skin pH in AD patients, causing an imbalance between serine proteases and protease inhibitors. Endogenous and exogenous proteases from house dust mites or *S.aureus* bacteria have increased activity in AD lesions, the lack of inhibitory activities of endogenous protease accelerates barrier permeability and inflammation, but increased pH and serine protamine levels reduce permeability and inflammation (Hachem *et al*., 2006).

Conclusions and Recommendations

Conclusions

1. AD may arise in people of any age, but with highest incidence rate for (5-15) years specifically.

2.The *Staphylococcus aureus* is the most common pathogen in the AD patients from lesional loci for all age group .

3.The study showed that an relationship between significant increase in inflammatory marker such as (IgE,IL-5 and IL-13) and AD disease.

4. The variance of serum level of inflammatory marker increased clearly in patients with AD and bacterial infection.

5. Total WBC counting of AD patients and most of lymphocytes, neutrophils and Eosinophils counting are abnormal in patients. Therefore, CBC test did reflect actual AD hitting

Recommendations

1. More studies are needed to determine the role of variation in other cytokines such as TLR in AD patients with and without bacterial infection .

2. Determine the antibacterial activity of some plant extract against bacterial isolation from AD patients in *vitro*.

3.More studies are needed to determine the effect of polymorphism of IL-13 and IL-5 in patients with and without bacterial infection.

Refrences

Reference

Akdis, C. A., Akdis, M., Trautmann, A., & Blaser, K. (2000). Immune regulation in atopic dermatitis. Current opinion in immunology, 12(6), 641-646.

Al-Ghanmi,H.(2016). Haematological indices and iron status among patients with renal failure in Babylon Governorate.(Master Degree in Scince),University of Kerbala, College of Scince.

Amat, F., Soria, A., Tallon, P., Bourgoin-Heck, M., Lambert, N., Deschildre, A., & Just, J. (2018). New insights into the phenotypes of atopic dermatitis linked with allergies and asthma in children: An overview. Clinical & Experimental Allergy, 48(8), 919-934.

Arikawa, J., Ishibashi, M., Kawashima, M., Takagi, Y., Ichikawa, Y., & Imokawa, G. (2002). Decreased levels of sphingosine, a natural antimicrobial agent, may be associated with vulnerability of the stratum corneum from patients with atopic dermatitis to colonization by *Staphylococcus aureus*. Journal of Investigative Dermatology, 119(2), 433-439.

Arkwright, P. D., Motala, C., Subramanian, H., Spergel, J., Schneider, L. C., & Wollenberg, A. (2013). Management of difficult-to-treat atopic dermatitis. The journal of allergy and clinical immunology: In Practice, 1(2), 142-151.

Bantz, S. K., Zhu, Z., & Zheng, T. (2014). The atopic march: progression from atopic dermatitis to allergic rhinitis and asthma. Journal of clinical & cellular immunology, 5(2).

Barnes, **K. C. (2010).** An update on the genetics of atopic dermatitis: scratching the surface in 2009. Journal of allergy and clinical immunology, 125(1), 16-29.

References

Baron, **E. J., Peterson, L. R. and Finegold, S. M. (1994).** Bailey and Scott's Diagnostic Microbiology. 9th Ed. The C.V. Mosby Company, U.S.A.

Beck, L. A., Thaçi, D., Hamilton, J. D., Graham, N. M., Bieber, T., Rocklin, R., & Radin, A. R. (2014). Dupilumab treatment in adults with moderate-to-severe atopic dermatitis. New England Journal of Medicine, 371(2), 130-139.

Bieber T (2008) .Atopic dermatitis . 358:1483_1.

Bieber, T. (2020). Interleukin-13: Targeting an underestimated cytokine in atopic dermatitis. Allergy, 75(1), 54-62.

Biedermann, T., Skabytska, Y., Kaesler, S., & Volz, T. (2015). Regulation of T cell immunity in atopic dermatitis by microbes: the Yin and Yang of cutaneous inflammation. Frontiers in immunology, 6, 353.

Bjerre, R. D., Bandier, J., Skov, L., Engstrand, L., & Johansen, J. D. (2017). The role of the skin microbiome in atopic dermatitis: a systematic review. British journal of dermatology, 177(5), 1272-1278.

Bonamonte, D., Filoni, A., Vestita, M., Romita, P., Foti, C., & Angelini, G. (2019). The role of the environmental risk factors in the pathogenesis and clinical outcome of atopic dermatitis. BioMed research international, 2019.

Boothe, W. D., Tarbox, J. A., & Tarbox, M. B. (2017). Atopic dermatitis: pathophysiology. In Management of Atopic Dermatitis (pp. 21-37). Springer, Cham.

Brandt, E. B., & Sivaprasad, U. (2011). Th2 cytokines and atopic dermatitis. Journal of clinical & cellular immunology, 2(3).

References

Brough, H. A., Nadeau, K. C., Sindher, S. B., Alkotob, S. S., Chan, S., Bahnson, H. T., & Lack, G. (2020). Epicutaneous sensitization in the development of food allergy: What is the evidence and how can this be prevented?. Allergy, 75(9), 2185-2205.

Brown, S. J., & McLean, W. I. (2012). One remarkable molecule: filaggrin. Journal of investigative dermatology, 132(3), 751-762.

Brunner, P. M., Silverberg, J. I., Guttman-Yassky, E., Paller, A. S., Kabashima, K., Amagai, M., ... & Taieb, A. (2017). Increasing comorbidities suggest that atopic dermatitis is a systemic disorder. Journal of Investigative Dermatology, 137(1), 18-25.

Bryld, L. E., Hindsberger, C., Kyvik, K. O., Agner, T., & Menné, T. (2003). Risk factors influencing the development of hand eczema in a population-based twin sample. British Journal of Dermatology, 149(6), 1214-1220 ISO 690.

Byrd, A. L., Deming, C., Cassidy, S. K., Harrison, O. J., Ng, W. I., Conlan, S., & NISC Comparative Sequencing Program. (2017). *Staphylococcus aureus* and *Staphylococcus epidermidis* strain diversity underlying pediatric atopic dermatitis. Science translational medicine, 9(397).

Cabanillas, B., & Novak, N. (2016). Atopic dermatitis and filaggrin. Current opinion in immunology, 42, 1-8.

Carolyn M W, Rose Z Hl, Jamie S-S, Jacques De, Emily C Brock, Natalie Kucirek, ZiadRifi, Jessica Wei, KarstenGronert Rachel B Brem, Gregory M Barton, Diana M Bautista (2019). Neutrophils promote CXCR3-dependent itch in the development of atopic dermatitis eLife 8: e48448.p: 1-33.

References

Carroll, C. L., Balkrishnan, R., Feldman, S. R., Fleischer Jr, A. B., & Manuel, J. C. (2005). The burden of atopic dermatitis: impact on the patient, family, and society. Pediatric dermatology, 22(3), 192-199..

Casas, C., Ginisty, H., Alvarez-Georges, S., Viodé, C., Lejeune, O., Rouvrais, C., & Redoules, D. (2008). Molecular characterization of inflammation and *Staphylococcus aureus* colonization of involved skin of atopic dermatitis patients. Skin Pharmacology and Physiology, 21(5), 260-268.

Cevikbas, F., & Steinhoff, M. (2012). IL-33: a novel danger signal system in atopic dermatitis. Journal of Investigative Dermatology, 132(5), 1326-1329.

Chaplin, D. D. (2010). Overview of the immune response. Journal of Allergy and Clinical Immunology, 125(2), S3-S23.

Choi, S. J., Song, M. G., Sung, W. T., Lee, D. Y., Lee, J. H., Lee, E. S., & Yang, J. M. (2003). Comparison of transepidermal water loss, capacitance and pH values in the skin between intrinsic and extrinsic atopic dermatitis patients. Journal of Korean Medical Science, 18(1), 93**.**

Chung, Y. M., Kim, S., Kim, N. I., Lee, E. Y., & Choue, R. (2005). Study of dietary fatty acids, blood fatty acid composition, and immune parameters in atopic dermatitis patients. Journal of nutrition and health, 38(7), 521-532.

Collee,J.G.; Fraser,A.G.; Mjarmion, B.P. and Simmons, A. (1996). Mackie and McCartney paractical medical microbiology. (14th ed.) Chrchill. Livingston.USA.

Cork, M. J., Danby, S. G., & Ogg, G. S. (2020). Atopic dermatitis epidemiology and unmet need in the United Kingdom. Journal of Dermatological Treatment, 31(8), 801-809.

References

Cork, M. J., Robinson, D. A., Vasilopoulos, Y., Ferguson, A., Moustafa, M., MacGowan, A., & Tazi-Ahnini, R. (2006). New perspectives on epidermal barrier dysfunction in atopic dermatitis: gene–environment interactions. Journal of Allergy and Clinical Immunology, 118(1), 3-21

Darsow, U., Wollenberg, A., Simon, D., Taïeb, A., Werfel, T., Oranje, A., ... & Giusti, F. (2013). Difficult to control atopic dermatitis. World Allergy Organization Journal, 6(1), 1-5.

Dekio, I., Hayashi, H., Sakamoto, M., Kitahara, M., Nishikawa, T., Suematsu, M., & Benno, Y. (2005). Detection of potentially novel bacterial components of the human skin microbiota using culture-independent molecular profiling. Journal of medical microbiology, 54(12), 1231-1238

Deleanu, D., & Nedelea, I. (2019). Biological therapies for atopic dermatitis: An update. Experimental and therapeutic medicine, 17(2), 1061-1067.

Dharmage, S. C., Lowe, A. J., Matheson, M. C., Burgess, J. A., Allen, K. J., & Abramson, M. J. (2014). Atopic dermatitis and the atopic march revisited. Allergy, 69(1), 17-27.

Egawa, G., & Kabashima, K. (2016). Multifactorial skin barrier deficiency and atopic dermatitis: essential topics to prevent the atopic march. Journal of Allergy and Clinical Immunology, 138(2), 350-358.

Eichenfield, L. F., Ellis, C. N., Mancini, A. J., Paller, A. S., & Simpson, E. L. (2012) . Atopic dermatitis: epidemiology and pathogenesis update. In Seminars in cutaneous medicine and surgery (. 31. 3,. S3-S5). WB Saunders.

References

Eichenfield, L. F., Hanifin, J. M., Beck, L. A., Lemanske, R. F., Sampson, H. A., Weiss, S. T., & Leung, D. Y. (2003). Atopic dermatitis and asthma: parallels in the evolution of treatment. *Pediatrics*, *111*(3), 608-616.

Geoghegan, J. A., Irvine, A. D., & Foster, T. J. (2018). *Staphylococcus aureus* and atopic dermatitis: a complex and evolving relationship. Trends in microbiology, 26(6), 484-497 ISO 690.

Goldman, E. and Green, L.H. (2015). Practical Handbook of Microbiology, Third Edition. CRC Press. Pp:50-85.

Gong, J. Q., Lin, L., Lin, T., Hao, F., Zeng, F. Q., Bi, Z. G., ... & Zhao, B. (2006). Skin colonization by *Staphylococcus aureus* in patients with eczema and atopic dermatitis and relevant combined topical therapy: a double-blind multicentre randomized controlled trial. British Journal of Dermatology, 155(4), 680-687.

Gooderham, M. J., Hong, H. C. H., Eshtiaghi, P., & Papp, K. A. (2018). Dupilumab: a review of its use in the treatment of atopic dermatitis. Journal of the American Academy of Dermatology, 78(3), S28-S36.

Gray, N. A., Dhana, A., Stein, D. J., & Khumalo, N. P. (2019). Zinc and atopic dermatitis: a systematic review and meta-analysis. Journal of the European Academy of dermatology and venereology, *33*(6), 1042-1050.

Greenfeder, S., Umland, S. P., Cuss, F. M., Chapman, R. W., & Egan, R. W. (2001). Th2 cytokines and asthma the role of interleukin-5 in allergic eosinophilic disease. Respiratory research, 2(2), 1-9.

Guttman-Yassky, E., Blauvelt, A., Eichenfield, L. F., Paller, A. S., Armstrong, A. W., Drew, J., & Simpson, E. L. (2020). Efficacy and safety of lebrikizumab, a

high-affinity interleukin 13 inhibitor, in adults with moderate to severe atopic dermatitis: a phase 2b randomized clinical trial. JAMA dermatology, 156(4), 411-420.

Hachem, J. P., Wagberg, F., Schmuth, M., Crumrine, D., Lissens, W., Jayakumar, A., & Egelrud, T. (2006). Serine protease activity and residual LEKTI expression determine phenotype in Netherton syndrome. Journal of Investigative Dermatology, 126(7), 1609-1621.

Halim, T. Y., Steer, C. A., Mathä, L., Gold, M. J., Martinez-Gonzalez, I., McNagny, K. M., & Takei, F. (2014). Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. immunity, 40(3), 425-435.

Hamann, C. R., & Thyssen, J. P. (2018). Monoclonal antibodies against interleukin 13 and interleukin 31RA in development for atopic dermatitis. Journal of the American academy of dermatology, 78(3), S37-S42.

Hello M,Aubert H, Bernier C, Neel A,Barbarot S. (2016) :Atopic dermatitis of the adult.Rev Med Interne ,37:91_9.

Henderson, J., Northstone, K., Lee, S. P., Liao, H., Zhao, Y., Pembrey, M., ... & Irvine, A. D. (2008). The burden of disease associated with filaggrin mutations: a population-based, longitudinal birth cohort study. Journal of Allergy and Clinical Immunology, 121(4), 872-877.

Hirasawa, Y., Takai, T., Nakamura, T., Mitsuishi, K., Gunawan, H., Suto, H., & Ogawa, H. (2009). *Staphylococcus aureus* extracellular protease causes epidermal barrier dysfunction. The Journal of investigative dermatology, 130(2), 614-617.

References

Hoare, C., Po, A. L. W., & Williams, H. (2000). Systematic Review of Treatments for Atopic Eczema. Health Technology Assessment , 4(37), 1.

Honda, T., Uehara, T., Matsumoto, G., Arai, S., & Sugano, M. (2016). Neutrophil left shift and white blood cell count as markers of bacterial infection. Clinica Chimica Acta, 457, 46-53.

Hong, S., Son, D. K., Lim, W. R., Kim, S. H., Kim, H., Yum, H. Y., & Kwon, H. (2012). The prevalence of atopic dermatitis, asthma, and allergic rhinitis and the comorbidity of allergic diseases in children. Environmental health and toxicology, 27.

Hummelshoj, T., Bodtger, U., Datta, P., Malling, H. J., Oturai, A., Poulsen, L. K., & Svejgaard, A. (2003). Association between an interleukin-13 promoter polymorphism and atopy. European journal of immunogenetics, 30(5), 355-359**.**

Hussein, Y. M., Ahmad, A. S., Ibrahem, M. M., Elsherbeny, H. M., Shalaby, S. M., El-Shal, A. S., & Sabbah, N. A. (2011). Interleukin 13 receptors as biochemical markers in atopic patients. Journal of investigational allergology & clinical immunology, 21(2), 101-107.

Illi, S., von Mutius, E., Lau, S., Nickel, R., Grüber, C., Niggemann, B., ... & Multicenter Allergy Study Group. (2004). The natural course of atopic dermatitis from birth to age 7 years and the association with asthma. Journal of Allergy and Clinical Immunology, 113(5), 925-931.

Ishimine, N., Honda, T., Yoshizawa, A., Kawasaki, K., Sugano, M., Kobayashi, Y., & Matsumoto, T. (2013). Combination of white blood cell count and Left Shift Level Real-Timely Reflects a Course of Bacterial Infection. Journal of clinical laboratory analysis, 27(5), 407-411.

References

James, W. D., Elston, D., & Berger, T. (2011). Andrew's Diseases of the Skin E-Book: Clinical Dermatology. Elsevier Health Sciences.

Janssens, M., van Smeden, J., Gooris, G. S., Bras, W., Portale, G., Caspers, P. J., ... & Lavrijsen, A. P. (2012). Increase in short-chain ceramides correlates with an altered lipid organization and decreased barrier function in atopic eczema patients. Journal of lipid research, 53(12), 2755-2766.

Jenerowicz, D., Czarnecka-O, M., & Silny, W. (2007). Peripheral blood eosinophilia in atopic dermatitis. Acta dermatovenerlogica Alpina panonica Et Adriatica , 16(2), 47.

Jinnestål, C. L., Belfrage, E., Bäck, O., Schmidtchen, A., & Sonesson, A. (2014). Skin barrier impairment correlates with cutaneous *staphylococcus aureus* colonization and sensitization to skin-associated microbial antigens in adult patients with atopic dermatitis. International journal of dermatology, 53(1), 27-33

Kasperkiewicz, M., Schmidt, E., Ludwig, R. J., & Zillikens, D. (2018). Targeting IgE antibodies by immunoadsorption in atopic dermatitis. Frontiers in immunology, 9, 254.

Kateete, D. P., Kimani, C. N., Katabazi, F. A., Okeng, A., Okee, M. S., Nanteza, A., ... & Najjuka, F. C. (2010). Identification of *Staphylococcus aureus*: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. Annals of clinical microbiology and antimicrobials, 9(1), 1-7.

Kijima, A., Murota, H., Takahashi, A., Arase, N., Yang, L., Nishioka, M., ... & Katayama, I. (2013). Prevalence and impact of past history of food allergy in atopic dermatitis. Allergology International, 62(1), 105-112 ISO 690.

References

Kim, J., Kim, B. E., & Leung, D. Y. (2019, March). Pathophysiology of atopic dermatitis: clinical implications. In Allergy and asthma proceedings :84.

Kobayashi T, Glatz M, Horiuchi K, Kawasaki H, Akiyama H, Kaplan DH, Kong HH, Amagai M, Nagao K. (2015). Dysbiosis and *Staphylococcus aureus* Colonization Drives Inflammation in atopic dermatitis. Immunity. 21;42(4):756-66. doi: 10.1016/j.immuni.2015.03.014. PMID: 25902485; PMCID: PMC4407815.

Kong, H. H., Oh, J., Deming, C., Conlan, S., Grice, E. A., Beatson, M. A., ... & Turner, M. L. (2012). Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. Genome research, 22(5), 850-859.

Kozo Nakai, Yasuo Kubota, and Chie Kohchi (2019). The effect of lipopolysaccharide-containing moisturizing Cream on Skin Care in Patients with Mild Atopic Dermatitis. 33(1), 109-114.

Krakowski, A. C., Eichenfield, L. F., & Dohil, M. A. (2008). Management of atopic dermatitis in the pediatric population. Pediatrics, 122(4), 812-824.

Kuczynski, J., Lauber, C. L., Walters, W. A., Parfrey, L. W., Clemente, J. C., Gevers, D., & Knight, R. (2012). Experimental and analytical tools for studying the human microbiome. Nature Reviews Genetics, 13(1), 47-58.

Kuo, I. H., Yoshida, T., De Benedetto, A., & Beck, L. A. (2013). The cutaneous innate immune response in patients with atopic dermatitis. Journal of allergy and clinical immunology, 131(2), 266-278.

References

Lee, H. J., & Lee, S. H. (2014). Epidermal permeability barrier defects and barrier repair therapy in atopic dermatitis. Allergy, asthma & immunology research, 6(4), 276.

Leung, D. Y., Boguniewicz, M., Howell, M. D., Nomura, I., & Hamid, Q. A. (2004). New insights into atopic dermatitis. The Journal of clinical investigation, 113(5), 651-657.

Li, Q., Yang, Y., Chen, R., Kan, H., Song, W., Tan, J., ... & Xu, J. (2016). Ambient air pollution, meteorological factors and outpatient visits for eczema in Shanghai, China: a time-series analysis. International journal of environmental research and public health, 13(11), 1106.

Liang, H. E., Reinhardt, R. L., Bando, J. K., Sullivan, B. M., Ho, I. C., & Locksley, R. M. (2012). Divergent expression patterns of IL-4 and IL-13 define unique functions in allergic immunity. Nature immunology, 13(1), 58.

Lin, Y. T., Wang, C. T., & Chiang, B. L. (2007). Role of bacterial pathogens in atopic dermatitis. Clinical reviews in allergy & immunology, 33(3), 167-177.

Liu, F. T., Goodarzi, H., & Chen, H. Y. (2011). IgE, mast cells, and eosinophils in atopic dermatitis. Clinical reviews in allergy & immunology, 41(3), 298-310.

Mailhol, C., Lauwers-Cances, V., Rancé, F., Paul, C., & Giordano-Labadie, F. (2009). Prevalence and risk factors for allergic contact dermatitis to topical treatment in atopic dermatitis: a study in 641 children. Allergy, 64(5), 801-806.

Maintz, L., & Novak, N. (2011). Modifications of the innate immune system in atopic dermatitis. Journal of innate immunity, 3(2), 131-141.

References

Marsella, R., Olivry, T., Carlotti, D. N., & International Task Force on Canine Atopic Dermatitis. (2011). Current evidence of skin barrier dysfunction in human and canine atopic dermatitis. Veterinary dermatology, *22*(3), 239-248.

Martinez-N.(2011). Rocio T., Fethi Louafi, and Tilman Sanchez-Elsner. "The interleukin 13 (IL-13) pathway in human macrophages is modulated by microRNA-155 via direct targeting of interleukin 13 receptor α1 (IL13Rα1)." Journal of biological chemistry 286. 3 :1786-1794.

Matsui, T., & Amagai, M. (2015). Dissecting the formation, structure and barrier function of the stratum corneum. International immunology, 27(6), 269-280. **Taskapan, M. O. & Kumar, P. (2000)** Role of staphylococcal superantigens in atopic dermatitis: from colonization to inflammation. Annals of allergy, asthma & immunology: official publication of the American College of Allergy, Asthma, & Immunology 84, 3–10; quiz 11–12, doi:10.1016/S1081-1206(10)62731-7).

McKenzie, C., & Silverberg, J. I. (2019). The prevalence and persistence of atopic dermatitis in urban United States children. Annals of Allergy, Asthma & Immunology, 123(2), 173-178.

Molfino, N. A., Gossage, D., Kolbeck, R., Parker, J. M., & Geba, G. P. (2012). Molecular and clinical rationale for therapeutic targeting of interleukin-5 and its receptor. Clinical & Experimental Allergy, 42(5), 712-737.

Moy, A. P., Murali, M., Kroshinsky, D., Duncan, L. M., & Nazarian, R. M. (2015). Immunologic overlap of helper T-cell subtypes 17 and 22 in erythrodermic psoriasis and atopic dermatitis. Jama Dermatology, 151(7), 753-760.

Mrabet-Dahbi, S., & Maurer, M. (2011). Innate immunity in atopic dermatitis. In Pathogenesis and Management of Atopic Dermatitis (Vol. 41, pp. 104-111).

References

Mu, Z., & Zhang, J. (2020). The role of genetics, the environment, and epigenetics in atopic dermatitis. In Epigenetics in Allergy and Autoimmunity (pp. 107-140). Springer, Singapore.

Naik, S., Bouladoux, N., Wilhelm, C., Molloy, M. J., Salcedo, R., Kastenmuller, W., ... & Spencer, S. (2012). Compartmentalized control of skin immunity by resident commensals. Science, 337(6098), 1115-1119.

Nakatsuji, T., Chen, T. H., Narala, S., Chun, K. A., Two, A. M., Yun, T., ... & Latif, H. (2017). Antimicrobials from human skin commensal bacteria protect against Staphylococcus aureus and are deficient in atopic dermatitis. Science translational medicine, 9(378).

Niebuhr, M., & Werfel, T. (2010). Innate immunity, allergy and atopic dermatitis. Current opinion in allergy and clinical immunology, 10(5), 463-468.

Nikhil D, Mayte S-F, Judilyn F-D, Julia K. Gittler, AvnerShemer, A, Vincent A. Fischetti, J G. Krueger, and Emma G- (2013). Attenuated neutrophil axis in atopic dermatitis compared to psoriasis reflects TH17 pathway differences between these diseases. J Allergy Clin.Immunol.; 132(2):498-501

Noda, S., Suárez-Fariñas, M., Ungar, B., Kim, S. J., de Guzman Strong, C., Xu, H., & Guttman-Yassky, E. (2015). The Asian atopic dermatitis phenotype combines features of atopic dermatitis and psoriasis with increased TH17 polarization. Journal of Allergy and Clinical Immunology, 136(5), 1254-1264.

Nomura, T., Akiyama, M., Sandilands, A., Nemoto-Hasebe, I., Sakai, K., Nagasaki, A., ... & Shimizu, H. (2008). Specific filaggrin mutations cause ichthyosis vulgaris and are significantly associated with atopic dermatitis in Japan. Journal of Investigative Dermatology, 128(6), 1436-1441.

References

Novak N, Bieber T, Kraft S (2004). Immunoglobulin E-bearing antigen-presenting cells in atopic dermatitis. Curr allergy asthma Rep 4(4):263–269

Nowicka, D., & Grywalska, E. (2018). The role of immune defects and colonization of *Staphylococcus aureus* in the pathogenesis of atopic dermatitis. Analytical Cellular Pathology.

Nutten, S. (2015). Atopic dermatitis: global epidemiology and risk factors. Annals of nutrition and metabolism, 66(Suppl. 1), 8-16.

Nyrén, M., Lindberg, M., Stenberg, B., Svensson, M., Svensson, Å., & Meding, B. (2005). Influence of childhood atopic dermatitis on future worklife. Scandinavian journal of work, environment & health, 474-478.

Obara, W., Kawa, Y., Ra, C., Nishioka, K., Soma, Y., & Mizoguchi, M. (2002). T cells and mast cells as a major source of interleukin-13 in atopic dermatitis. Dermatology, 205(1), 11-17**.**

Ong, P. Y., & Leung, D. Y. (2016). Bacterial and viral infections in atopic dermatitis: a comprehensive review. Clinical reviews in allergy & immunology, 51(3), 329-337.

ORegan, G. M., Sandilands, A., McLean, W. I., & Irvine, A. D. (2008). Filaggrin in atopic dermatitis. Journal of Allergy and Clinical Immunology, 122(4), 689-693.

Ortega, H. G., Liu, M. C., Pavord, I. D., Brusselle, G. G., FitzGerald, J. M., Chetta, A., & Chanez, P. (2014). Mepolizumab treatment in patients with severe eosinophilic asthma. New England Journal of Medicine, 371(13), 1198-1207.

References

Otsuka, A., Nomura, T., Rerknimitr, P., Seidel, J. A., Honda, T., & Kabashima, K. (2017). The interplay between genetic and environmental factors in the pathogenesis of atopic dermatitis. Immunological reviews, 278(1), 246-262.

Paller, A. S., Kong, H. H., Seed, P., Naik, S., Scharschmidt, T. C., Gallo, R. L., & Irvine, A. D. (2019). The microbiome in patients with atopic dermatitis. Journal of Allergy and Clinical Immunology, 143(1), 26-35.

Park, C. O., Noh, S., Jin, S., Lee, N. R., Lee, Y. S., Lee, H., & Lee, K. H. (2013). Insight into newly discovered innate immune modulation in atopic dermatitis. Experimental dermatology, 22(1), 6-9.

Pellefigues, C. (2020). IgE autoreactivity in atopic dermatitis: Paving the Road for Autoimmune Diseases. Antibodies, 9(3), 47.

Peng W and Novak N (2015). Pathogenesis of atopic dermatitis. Clin exp allergy 45(3): 566-574.

Phipps, S., Ying, S., Wangoo, A., Ong, Y. E., Levi-Schaffer, F., & Kay, A. B. (2002). The relationship between allergen-induced tissue eosinophilia and markers of repair and remodeling in human atopic skin. The Journal of Immunology, 169(8), 4604-4612.

Pulendran, B., & Artis, D. (2012). New paradigms in type 2 immunity. Science, 337(6093), 431-435.

Pyun, B. Y. (2015). Natural history and risk factors of atopic dermatitis in children. Allergy, asthma & immunology research, 7(2), 101.

Raap, U., Werfel, T., Goltz, C., Deneka, N., Langer, K., Bruder, M., ... & Wedi, B. (2006). Circulating levels of brain-derived neurotrophic factor correlate

with disease severity in the intrinsic type of atopic dermatitis. Allergy, 61(12), 1416-1418.

Ramirez, F D., Shelley Ch, Sinéad M. L, Aric A. P, Charles E. Mc, Sharon A. K, Michael D. Cabana, Mary-Margaret Chren, and Katrina Abuabara. (2019)"Association of atopic dermatitis with sleep quality in children." JAMA pediatrics 173, no. 5: e190025-e190025.

Rebane, A., Runnel, T., Aab, A., Maslovskaja, J., Rückert, B., Zimmermann, M., ... & Haljasorg, U. (2014). MicroRNA-146a alleviates chronic skin inflammation in atopic dermatitis through suppression of innate immune responses in keratinocytes. Journal of allergy and clinical immunology, 134(4), 836-847.

Rippke, F., Schreiner, V., Doering, T., & Maibach, H. I. (2004). Stratum corneum pH in atopic dermatitis. American journal of clinical dermatology, 5(4), 217-223.

Rothenberg ME, Hogan SP (2006) The eosinophil. Annu Rev Immunol 24:147–174.

Sacco, K. A., & Milner, J. D. (2019). Gene–environment interactions in primary atopic disorders. Current opinion in immunology, 60, 148-155.

Sacotte, R., & Silverberg, J. I. (2018). Epidemiology of adult atopic dermatitis. Clinics in dermatology, 36(5), 595-605.

Salava, A., & Lauerma, A. (2014). Role of the skin microbiome in atopic dermatitis. Clinical and translational allergy, 4(1), 33

Sambrook, J. and Rusell, D. W. (2001). Molecular cloning. A laboratory manual. 3rd ed. Cold spring. Harber laboratory press. New York

References

Sampson A.B., Yousif E. and Hossain J. (2010). Evaluation of the relationship between IgE level and skin super infection in children with atopic dermatitis. Allergy Asthma Proc.; 31(3): 232-237.

Sathishkumar, D., & Moss, C. (2016). Topical therapy in atopic dermatitis in children. Indian journal of dermatology, 61(6), 656.

Saunders, S. P., Moran, T., Floudas, A., Wurlod, F., Kaszlikowska, A., Salimi, M., ... & Hams, E. (2016). Spontaneous atopic dermatitis is mediated by innate immunity, with the secondary lung inflammation of the atopic march requiring adaptive immunity. Journal of Allergy and Clinical Immunology, 137(2), 482-491.

Seghers, A. C., Lee, J. S., Tan, C. S., Koh, Y. P., Ho, M. S., Lim, Y. L., ... & Tang, M. B. (2014). Atopic dirty neck or acquired atopic hyperpigmentation? An epidemiological and clinical study from the National Skin Centre in Singapore. Dermatology, 229(3), 174-182.

Seo, E., Yoon, J., Jung, S., Lee, J., Lee, B. H., & Yu, J. (2019). Phenotypes of atopic dermatitis identified by cluster analysis in early childhood. The Journal of dermatology, 46(2), 117-123.

Shaw, T.E; Currie, G.P.; Koudelka, C.W.; Simpson, E.L. (2011) :Eczema prevalence in United states : Data from the 2003 National Survey of children s Health . J. Investing . Dermatol ; 131, 67_73.

Simon, D., Braathen, L. R., & Simon, H. U. (2004). Eosinophils and atopic dermatitis. Allergy, 59(6), 561-570.

Simon, D., Vassina, E., Yousefi, S., Kozlowski, E., Braathen, L. R., & Simon, H. U. (2004). Reduced dermal infiltration of cytokine-expressing inflammatory

cells in atopic dermatitis after short-term topical tacrolimus treatment. Journal of allergy and clinical immunology, 114(4), 887-895.

Simon, D., Wollenberg, A., Renz, H., & Simon, H. U. (2019). Atopic Dermatitis: Collegium Internationale Allergologicum (CIA) Update 2019. International archives of allergy and immunology, 178(3), 207-218.

Sirufo, M. M., De Martinis, M., & Ginaldi, L. (2018). Omalizumab an effective and safe alternative therapy in severe refractory atopic dermatitis: A case report. Medicine, 97(24).

Sonkoly, E., Muller, A., Lauerma, A. I., Pivarcsi, A., Soto, H., Kemeny, L., ... & Homey, B. (2006). IL-31: a new link between T cells and pruritus in atopic skin inflammation. Journal of Allergy and Clinical Immunology, 117(2), 411-417.

Soumelis, V., Reche, P. A., Kanzler, H., Yuan, W., Edward, G., Homey, B., ... & Smith, K. (2002). Human epithelial cells trigger dendritic cell–mediated allergic inflammation by producing TSLP. Nature immunology, 3(7), 673-680.

Spergel, J. M. (2010). Epidemiology of atopic dermatitis and atopic march in children. Immunology and Allergy Clinics, 30(3), 269-280.

Staumont-S, D., Fleury, S., Lazzari, A., Molendi-Coste, O., Hornez, N., Lavogiez, C and Mionnet, C. (2014). CX3CL1 (fractalkine) and its receptor CX3CR1 regulate atopic dermatitis by controlling effector T cell retention in inflamed skin. Journal of Experimental Medicine, 211(6), 1185-1196.

Suarez-Farinas, M., Dhingra, N., Gittler, J., Shemer, A., Cardinale, I., de Guzman Strong, C., ... & Guttman-Yassky, E. (2013). Intrinsic atopic dermatitis

shows similar TH2 and higher TH17 immune activation compared with extrinsic atopic dermatitis. Journal of Allergy and Clinical Immunology, 132(2), 361-370.

Sun, Z., Huang, S., Zhu, P., Yue, F., Zhao, H., Yang, M., ... & Callewaert, C. (2019). A microbiome-based index for assessing skin health and treatment effects for atopic dermatitis in children. Msystems, 4(4), e00293-19.

Taskapan, M. O., & Kumar, P. (2000). Role of staphylococcal superantigens in atopic dermatitis: from colonization to inflammation. Annals of Allergy, Asthma & Immunology, 84(1), 3-12.

Thomsen, S. F. (2014). Atopic dermatitis: natural history, diagnosis, and treatment. International Scholarly Research Notices.

Tokura, Y. (2010). Extrinsic and intrinsic types of atopic dermatitis. Journal of dermatological science, 58(1), 1-7.

Tsakok, T., Woolf, R., Smith, C. H., Weidinger, S., & Flohr, C. (2019). Atopic dermatitis: the skin barrier and beyond. British journal of dermatology, 180(3), 464-474.

Van Smeden, J., Janssens, M., Kaye, E. C., Caspers, P. J., Lavrijsen, A. P., Vreeken, R. J., & Bouwstra, J. A. (2014). The importance of free fatty acid chain length for the skin barrier function in atopic eczema patients. Experimental dermatology, 23(1), 45-52.

Vandepitte, J., Verhaegen, J., Engbaek, K., Piot, P., Heuck, C. C., Rohner, P., & Heuck, C. C. (2003). Basic laboratory procedures in clinical bacteriology. World Health Organization.

References

Vander Hulst, A. E., Klip, H., & Brand, P. L. (2007). Risk of developing asthma in young children with atopic eczema: a systematic review. Journal of Allergy and Clinical Immunology, 120(3), 565-569.

Wang, A-x, and Ning X. L. "New insights into T cells and their signature cytokines in atopic dermatitis." IUBMB life 67.8 (2015): 601-610.

Williams, H. C. (2000). Epidemiology of atopic dermatitis. Clinical and experimental dermatology, 25(7), 522-529.

Williams, M. R., & Gallo, R. L. (2015). The role of the skin microbiome in atopic dermatitis. Current allergy and asthma reports, 15(11), 65.

Wollenberg, A., & Bieber, T. (2000). Atopic dermatitis: from the genes to skin lesions. Allergy, 55(3), 205-213.

Wollenberg, A., & Feichtner, K. (2013). Atopic dermatitis and skin allergies–update and outlook. Allergy, 68(12), 1509-1519

Wollenberg, A., Oranje, A., Deleuran, M., Simon, D., Szalai, Z., Kunz, B., ... & de Bruin-Weller, M. (2016). ETFAD/EADV Eczema task force 2015 position paper on diagnosis and treatment of atopic dermatitis in adult and paediatric patients. Journal of the European Academy of Dermatology and Venereology, 30(5), 729-747.

Wollenberg, A., Räwer, H. C., & Schauber, J. (2011). Innate immunity in atopic dermatitis. Clinical reviews in allergy & immunology, 41(3), 272-281.

Yamamoto, N., Sugiura, H., Tanaka, K., & Uehara, M. (2003). Heterogeneity of interleukin 5 genetic background in atopic dermatitis patients: significant

difference between those with blood eosinophilia and normal eosinophil levels. Journal of dermatological science, 33(2), 121-126.

Yamanaka, K. I., & Mizutani, H. (2011). The role of cytokines/chemokines in the pathogenesis of atopic dermatitis. Pathogenesis and management of atopic dermatitis .

Yamazaki, Y., Nakamura, Y., & Núñez, G. (2017). Role of the microbiota in skin immunity and atopic dermatitis. Allergology International, 66(4), 539-544.

Ying Jiang and Wencong Ma. (2017). Assessment of neutrophil-to lymphocyte ratio and platelet-to-lymphocyte ratio in atopic dermatitis Patients. Med SciMonit. 23: 1340–1346**.**

Young B (2006) Wheater's functional histology: a text and colour atlas, 5th edn. Churchill Livingstone/Elsevier, Edinburgh, 437 .

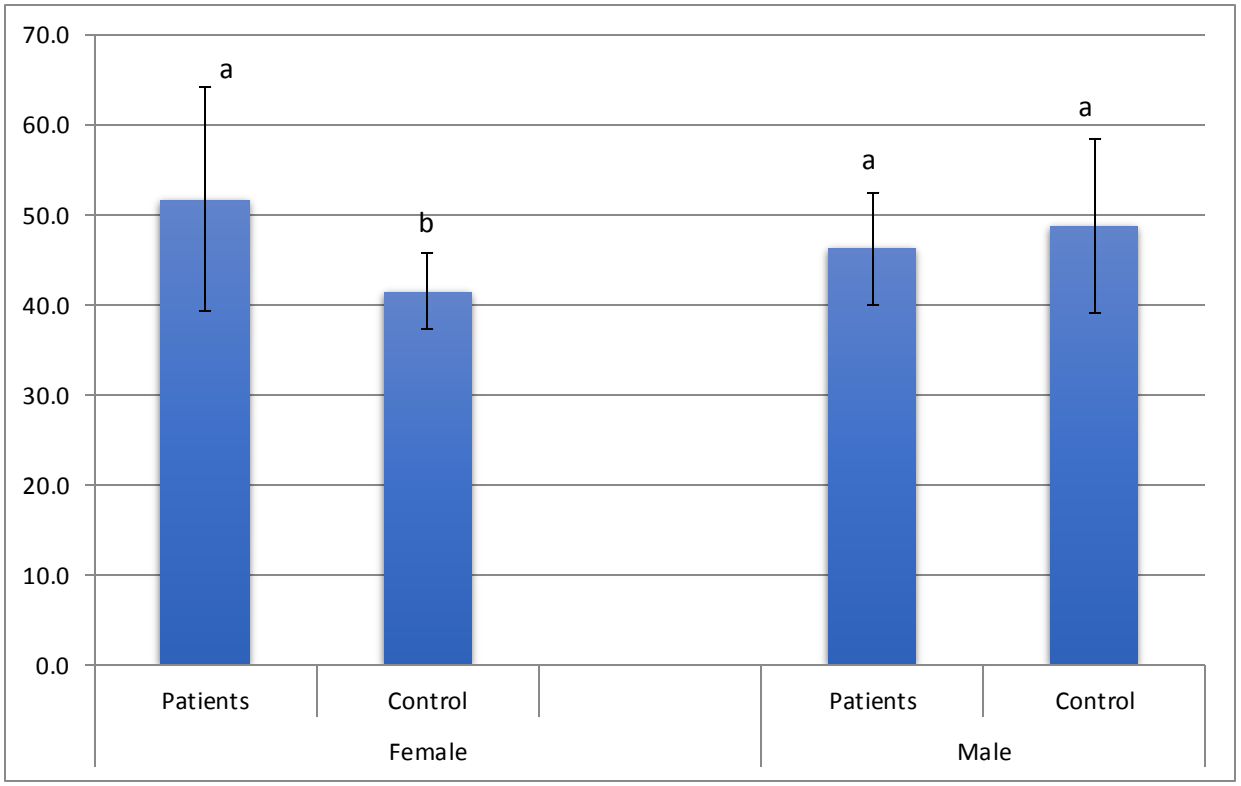
Zheng, T., Oh, M. H., Oh, S. Y., Schroeder, J. T., Glick, A. B., & Zhu, Z. (2009). Transgenic expression of interleukin-13 in the skin induces a pruritic dermatitis and skin remodeling. Journal of investigative dermatology, 129(3), 742-751.

Zheng, T., Yu, J., Oh, M. H., & Zhu, Z. (2011). The atopic march: progression from atopic dermatitis to allergic rhinitis and asthma. Allergy, asthma and immunology research, 3(2), 67.

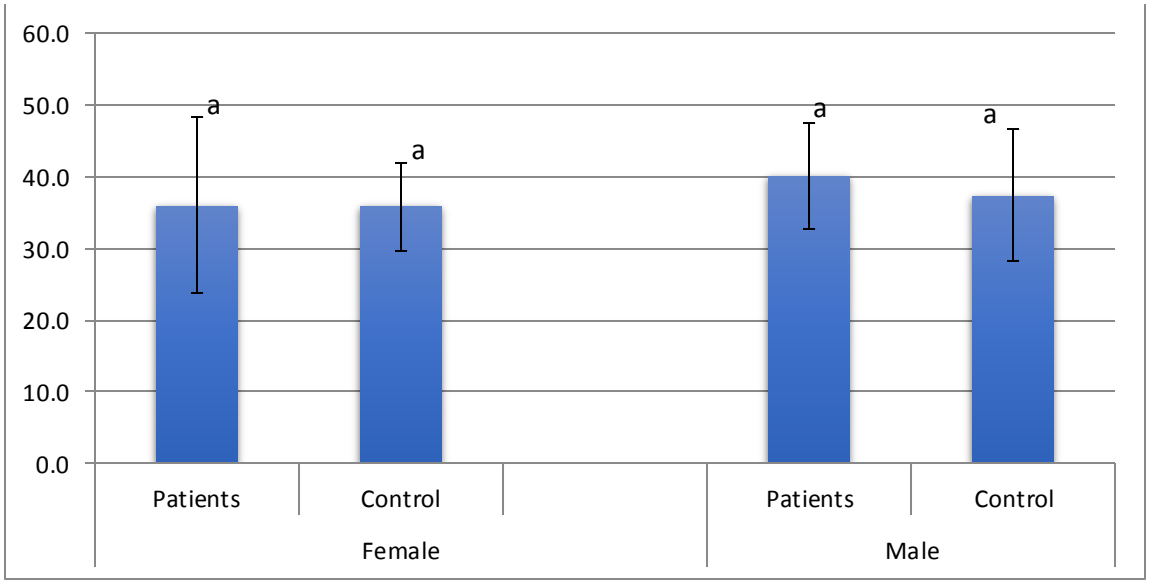
Zina M. Yahya (2021).Some bacteriological and immunological aspects of microbiota effect in atopic dermatitis and healthy Iraqi individuals , MSc Thesis ,College of Sciences, University of Baghdad .

Appendix

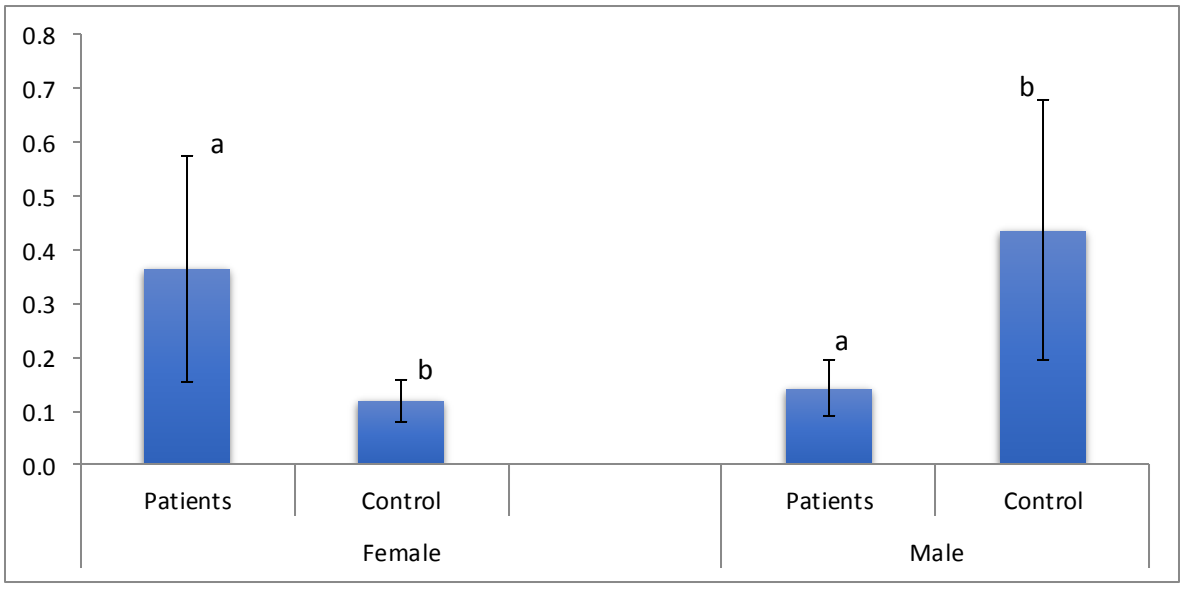
Appendix(1): Number of Neutrophils from patients and control groups of female and male. Data are presented as mean ± SD in each group of sex. Columns with different letters in the same gender are significantly different ($P < 0.05$).



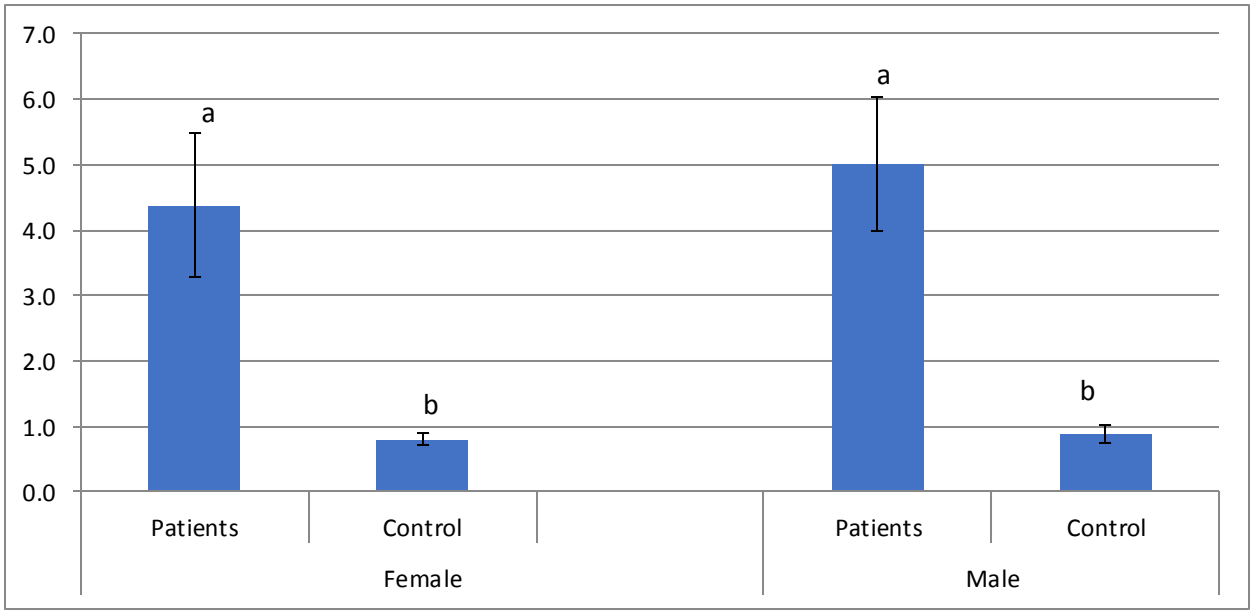
Appendix(2): Number of lymphocytes from patients and control groups of female and male. Data are presented as mean ± SD in each group of sex. Columns with different letters in the same gender are significantly different ($P < 0.05$).



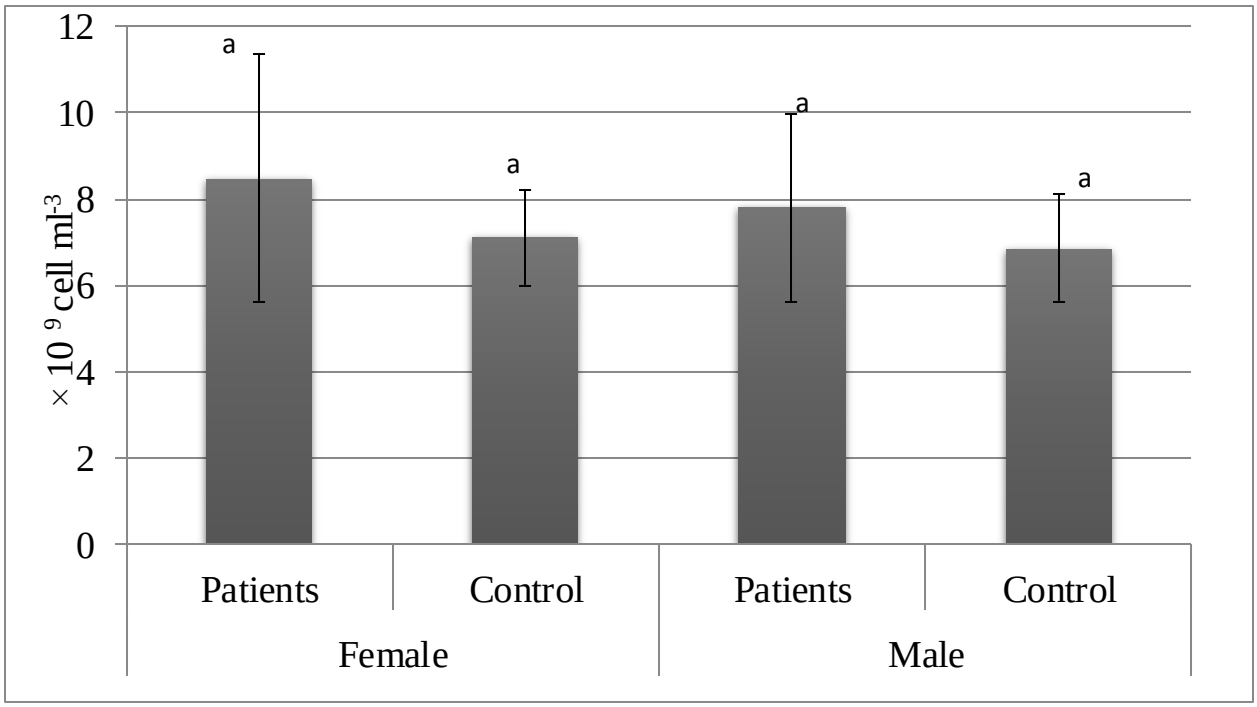
Appendix(3): Number of basophils from patients and control groups of female and male. Data are presented as mean ± SD in each group of sex. Columns with different letters in the same gender are significantly different ($P < 0.05$).



Appendix(4): Number of eosinophil from patients and control groups of female and male. Data are presented as mean ± SD in each group of sex. Columns with different letters in the same gender are significantly different ($P < 0.05$).



Appendix(5): Total white blood cell count in the studies groups. Data are presented as mean ± SD in each group of sex. Columns with different letters in the same gender are significantly different ($P < 0.05$).

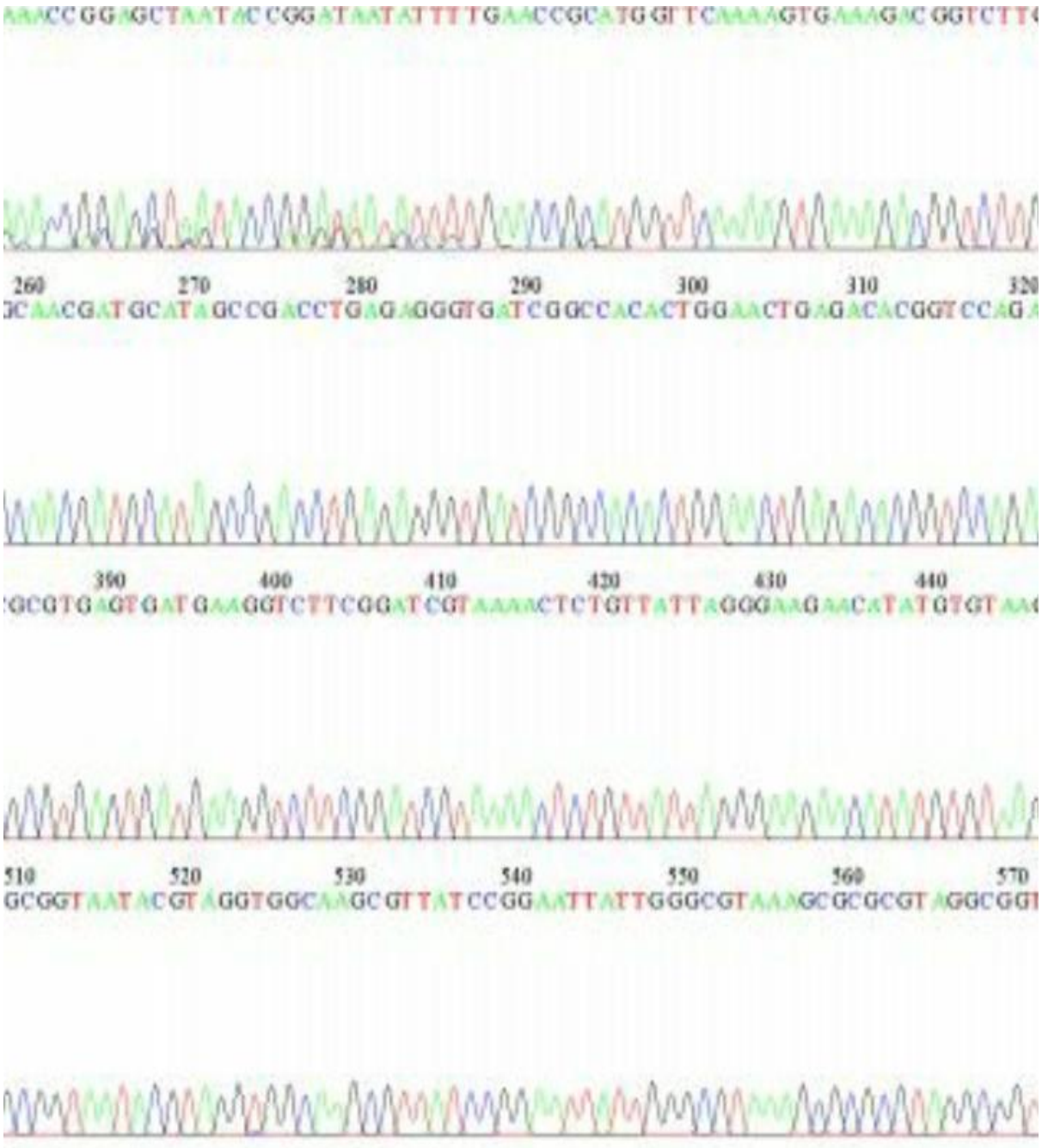


Appendix(6): DNA Sequence of *S.aureus* isolated from infected area of AD patients

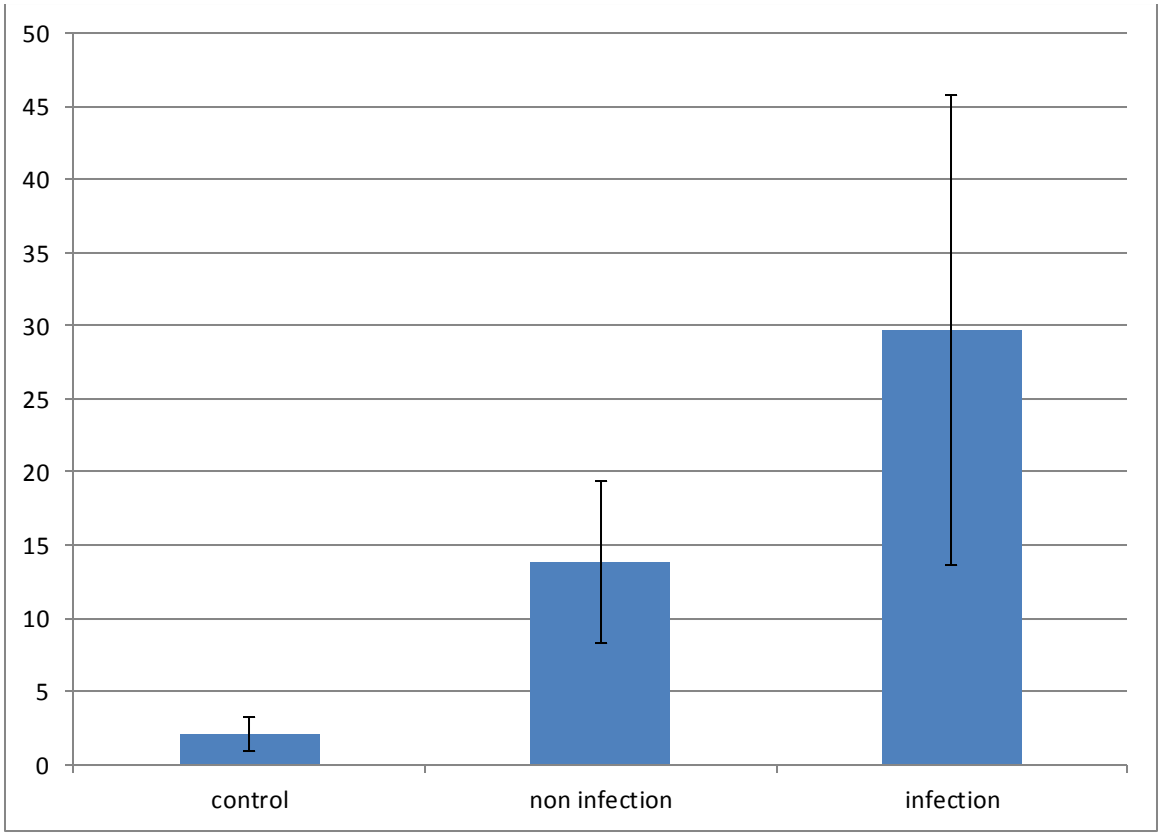
>H201006-065_A17_X1_XF.ab1 1157

CGTTAGGGGACCTTGAAAGGTACAGTGATACATGCTGATCGAGGCTCGGA
AGAGGAGCTTGGGTCTCTGATGTTAGAGGCGGATCGGGGAGTAACACGTG
GATAACCGACCTATAAGACTGGAATAACTTCGGGAAACCGGAGCTAATAC
CGGATAATATTTTGAACCGCATGGTTCAAAAGTGAAAGACGGTCTTGCTG
TCACTTATAGATGGATCCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGC
TTACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACT
GGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCT
TCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAGG
TCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAA
CTGTGCACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTG
CCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGG
GCGTAAAGCGCGCGTAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCACGG
CTCAACCGTGGAGGGTCATTGGAAACTGGAAAACTTGAGTGCAGAAGAGG
AAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGAAC
ACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAA
AGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAAC
GATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAAC
GCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAG
GAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAG
CAACGCGAAGAACCTTACCAAATCTTGACATCCTTTGACAACTCTGGAGA
TAGAGCTTTCCCCTTCGGGGGACAAAGTGACAGGTGGTGCATGGTTGTCG
TCAGCTCGGGTCGTGAGAAGTTGGGGTTAAGTCCCGCAACGAGGGCAACC
CTTTAAGCTTATTTGCCCTTATTTAATTTGGGCCCCTTTAAATTTGAACG
CCGGGGG

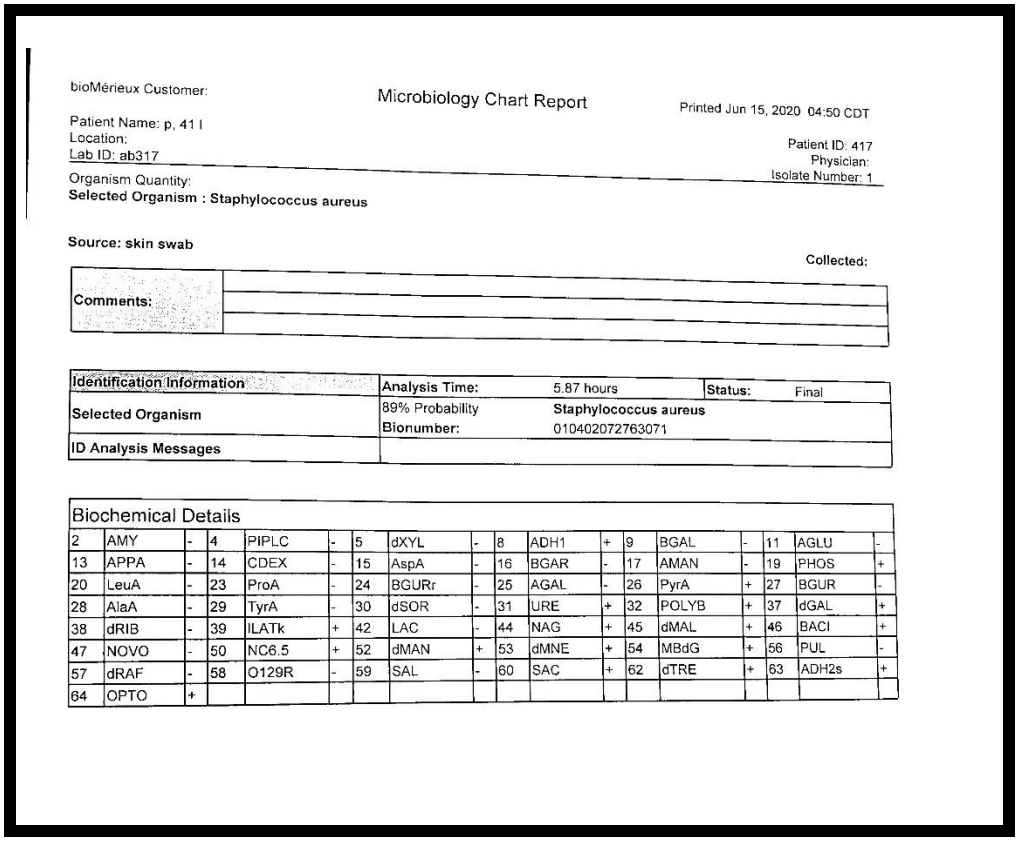
Appendix(7): Peaks for DNA sequence of *S.aureus* isolated from infected area of AD patients



Appendix(8): The relationship between level of IL13 ,IL5 and IgE in infected bacterial , non-infected bacterial AD patients and the control groups



Appendix(9): results of Viteks test for more common isolated bacteria



bioMérieux Customer:

Microbiology Chart Report

Printed Jun 15, 2020 04:50 CDT

Patient Name: p, 41 l
Location:
Lab ID: ab317

Patient ID: 417
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus

Source: skin swab

Collected:

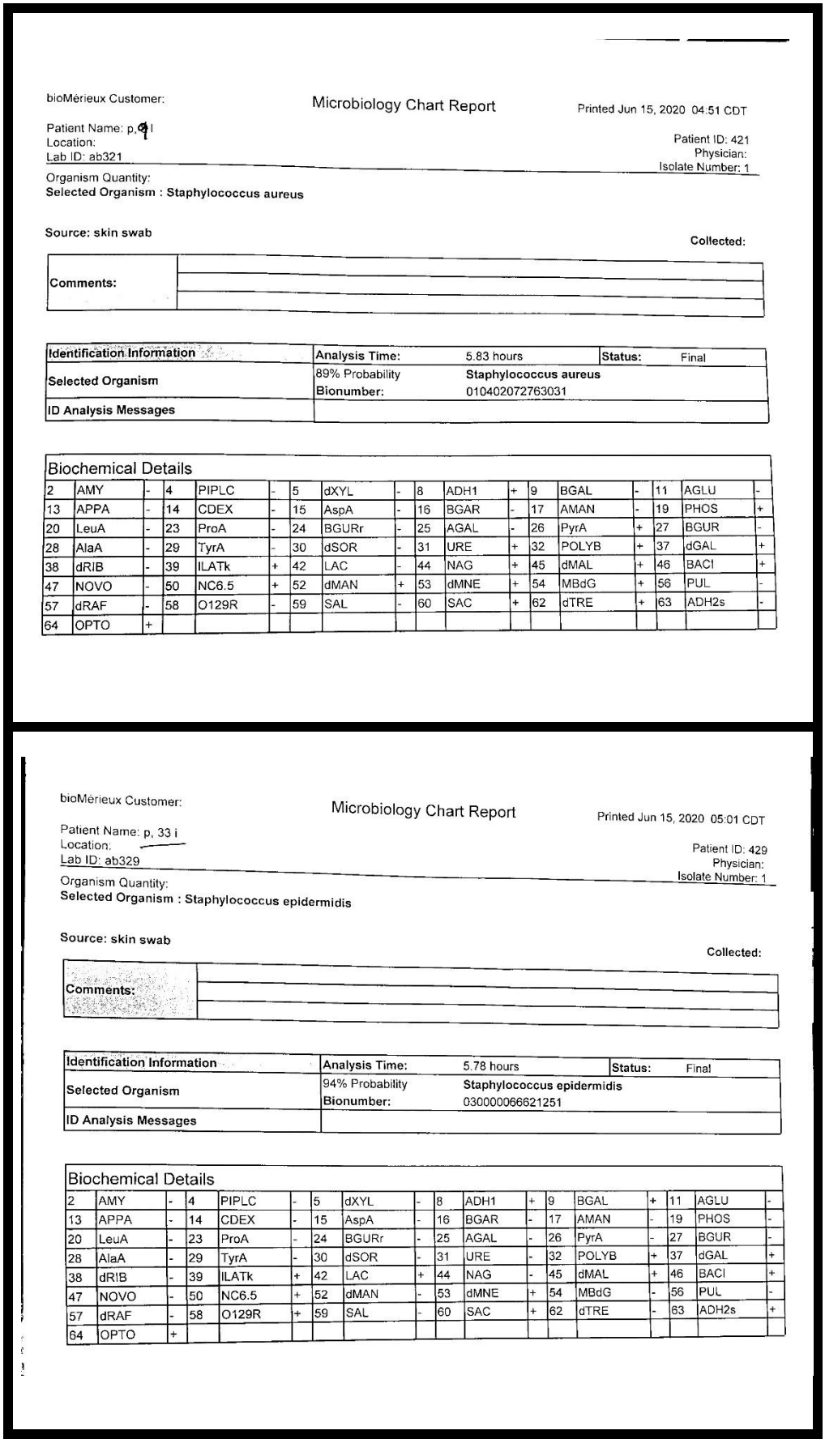
Comments:	

Identification Information	**Analysis Time:**	5.87 hours	**Status:**	Final
Selected Organism	89% Probability	**Staphylococcus aureus**		
	Bionumber:	010402072763071		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	+	37	dGAL	+
38	dRIB	-	39	ILATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	+
64	OPTO	+															

Appendix



bioMérieux Customer:

Microbiology Chart Report

Printed Jun 15, 2020 04:51 CDT

Patient Name: p, 9 i
Location:
Lab ID: ab321

Patient ID: 421
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus

Source: skin swab

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.83 hours	**Status:**	Final
Selected Organism	89% Probability **Bionumber:**	**Staphylococcus aureus** 010402072763031		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	+	37	dGAL	+
38	dRIB	-	39	ILATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 15, 2020 05:01 CDT

Patient Name: p, 33 i
Location:
Lab ID: ab329

Patient ID: 429
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus epidermidis

Source: skin swab

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.78 hours	**Status:**	Final
Selected Organism	94% Probability **Bionumber:**	**Staphylococcus epidermidis** 030000066621251		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	+	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	-	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	+	37	dGAL	+
38	dRIB	-	39	ILATk	+	42	LAC	+	44	NAG	-	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	-	53	dMNE	+	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	-	63	ADH2s	+
64	OPTO	+															

Appendix

bioMerieux Customer:

Microbiology Chart Report

Printed Jun 15, 2020 04:52 CDT

Patient Name: p, 1 i infected
Location:
Lab ID: ab322

Patient ID: 422
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus

Source: skin swab

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.83 hours	**Status:**	Final
Selected Organism	89% Probability **Bionumber:**	**Staphylococcus aureus** 010402072763031		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-		
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+		
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-		
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	+	37	dGAL	+		
38	dRIB	-	39	ILATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+		
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-		
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-		
64	OPTO	+																	

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 15, 2020 04:58 CDT

Patient Name: p, 12 i infected
Location:
Lab ID: ab325

Patient ID: 425
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus

Source: skin swab

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.82 hours	**Status:**	Final
Selected Organism	89% Probability **Bionumber:**	**Staphylococcus aureus** 010402072763031		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-		
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+		
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-		
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	+	37	dGAL	+		
38	dRIB	-	39	ILATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+		
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-		
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-		
64	OPTO	+																	

Appendix

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 15, 2020 04:59 CDT

Patient Name: p, 33 l Local
Location:
Lab ID: ab328

Patient ID: 428
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus haemolyticus

Source: skin swab

Collected:

Comments:	

Identification Information	**Analysis Time:**	4.82 hours	**Status:**	Final
Selected Organism	99% Probability **Bionumber:**	**Staphylococcus haemolyticus** 010002046720271		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	(-)
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	-	37	dGAL	+
38	dRIB	-	39	ILATk	+	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	-	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	+
64	OPTO	+															

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 15, 2020 04:51 CDT

Patient Name: p, 50 i
Location:
Lab ID: ab319

Patient ID: 419
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus

Source: skin swab

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.85 hours	**Status:**	Final
Selected Organism	89% Probability **Bionumber:**	**Staphylococcus aureus** 010402072763031		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	+	37	dGAL	+
38	dRIB	-	39	iLATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Appendix

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 15, 2020 05:02 CDT

Patient Name: 2, c Control من ضمن ال 30 Control
Location:
Lab ID: ab331

Patient ID: 431
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus epidermidis

Source: skin swab

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.03 hours	**Status:**	Final
Selected Organism	99% Probability **Bionumber:**	**Staphylococcus epidermidis** 010000076620211		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	-	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	+	37	dGAL	+
38	dRIB	-	39	ILATk	+	42	LAC	+	44	NAG	-	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	-	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	-	63	ADH2s	-
64	OPTO	+															

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 15, 2020 04:52 CDT

Patient Name: p, 1 l
Location:
Lab ID: ab323

Patient ID: 423
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus

Source: skin swab

Collected:

Comments:	

Identification Information	**Analysis Time:**	4.85 hours	**Status:**	Final
Selected Organism	96% Probability **Bionumber:**	**Staphylococcus aureus** 050402072763031		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	+
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	+	37	dGAL	+
38	dRIB	-	39	ILATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Appendix

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 15, 2020 04:58 CDT

Patient Name: p, 20 i
Location:
Lab ID: ab327

Patient ID: 427
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus haemolyticus

Source: skin swab

Collected:

Comments:	

Identification Information	**Analysis Time:**	4.83 hours	**Status:**	Final
Selected Organism	99% Probability **Bionumber:**	**Staphylococcus haemolyticus** 010002046720231		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	-	37	dGAL	+
38	dRIB	-	39	ILATk	+	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	-	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 15, 2020 04:48 CDT

Patient Name: i, 14
Location:
Lab ID: ab314

Patient ID: 414
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus

Source: skin swab

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.90 hours	**Status:**	Final
Selected Organism	95% Probability **Bionumber:**	**Staphylococcus aureus** 010402072763271		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	(-)	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	+	37	dGAL	+
38	dRIB	(-)	39	iLATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	+
64	OPTO	+															

Appendix

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 15, 2020 05:01 CDT

Patient Name: p, 3 I
Location:
Lab ID: ab330

Patient ID: 430
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus haemolyticus

Source: skin swab

Collected:

Comments:	

Identification Information	**Analysis Time:**	4.80 hours	**Status:**	Final
Selected Organism	99% Probability	**Staphylococcus haemolyticus**		
	Bionumber:	0100020467202 31		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	-	37	dGAL	+
38	dRIB	-	39	ILATk	+	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	-	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 15, 2020 04:51 CDT

Patient Name: p[illegible] I
Location:
Lab ID: ab320

Patient ID: 420
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus

Source: skin swab

Collected:

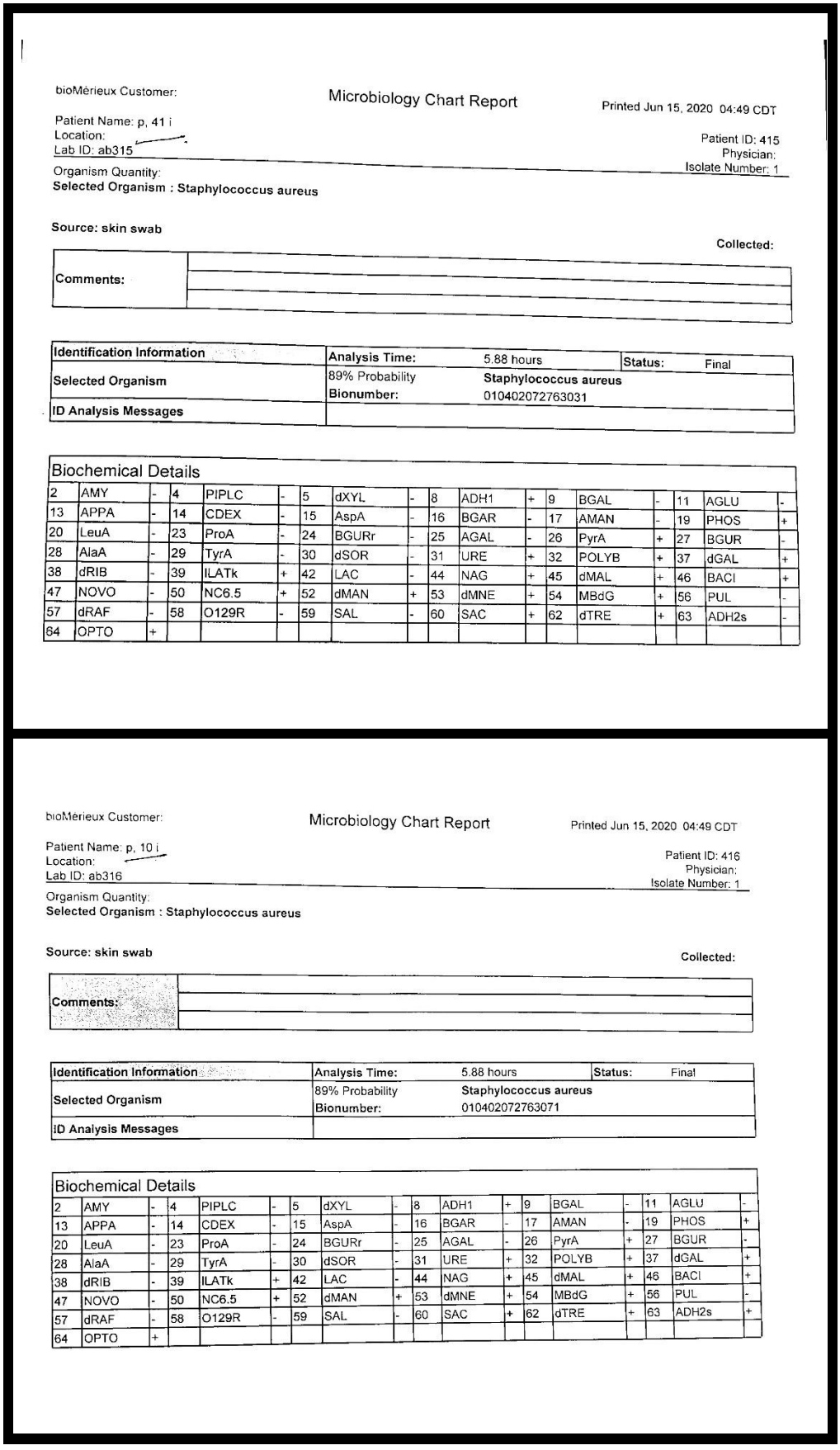
Comments:	Skin Swab from infected area

Identification Information	**Analysis Time:**	5.85 hours	**Status:**	Final
Selected Organism	89% Probability	**Staphylococcus aureus**		
	Bionumber:	010402072763031		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	+	37	dGAL	+
38	dRIB	-	39	ILATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Appendix



bioMérieux Customer:

Microbiology Chart Report

Printed Jun 15, 2020 04:49 CDT

Patient Name: p, 41 i
Location:
Lab ID: ab315

Patient ID: 415
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus

Source: skin swab

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.88 hours	**Status:**	Final
Selected Organism	89% Probability **Bionumber:**	**Staphylococcus aureus** 010402072763031		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	+	37	dGAL	+
38	dRIB	-	39	ILATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 15, 2020 04:49 CDT

Patient Name: p, 10 i
Location:
Lab ID: ab316

Patient ID: 416
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus

Source: skin swab

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.88 hours	**Status:**	Final
Selected Organism	89% Probability **Bionumber:**	**Staphylococcus aureus** 010402072763071		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	+	37	dGAL	+
38	dRIB	-	39	ILATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	+
64	OPTO	+															

Appendix

bioMèrieux Customer:

Microbiology Chart Report

Printed Jun 15, 2020 04:51 CDT

Patient Name: p, 10 l
Location:
Lab ID: ab318

Patient ID: 418
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus

Source: skin swab

Collected:

Comments:	Skin Swab from Local

Identification Information	**Analysis Time:**	5.87 hours	**Status:**	Final
Selected Organism	89% Probability **Bionumber:**	**Staphylococcus aureus** 010402072763071		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	+	37	dGAL	+
38	dRIB	-	39	ILATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	+
64	OPTO	+															

bioMèrieux Customer:

Microbiology Chart Report

Printed Jun 15, 2020 04:58 CDT

Patient Name: p, 32 l Local
Location:
Lab ID: ab324

Patient ID: 424
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus

Source: skin swab

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.83 hours	**Status:**	Final
Selected Organism	90% Probability **Bionumber:**	**Staphylococcus aureus** 010402072763071		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	+	37	dGAL	+
38	dRiB	(-)	39	ILATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	+
64	OPTO	+															

Appendix

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 22, 2020 04:50 CDT

Patient Name: p, 2i
Location:
Lab ID: ab333

Patient ID: 433
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Pseudomonas stutzeri

Source:

Collected:

Comments:	

Identification Information	**Analysis Time:**	7.82 hours	**Status:**	Final
Selected Organism	95% Probability **Bionumber:**	**Pseudomonas stutzeri** 0003011100600000		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	-	41	AGLU	+	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 22, 2020 04:48 CDT

Patient Name: 4, i
Location:
Lab ID: ab332

Patient ID: 432
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Kocuria rhizophila

Source:

Collected:

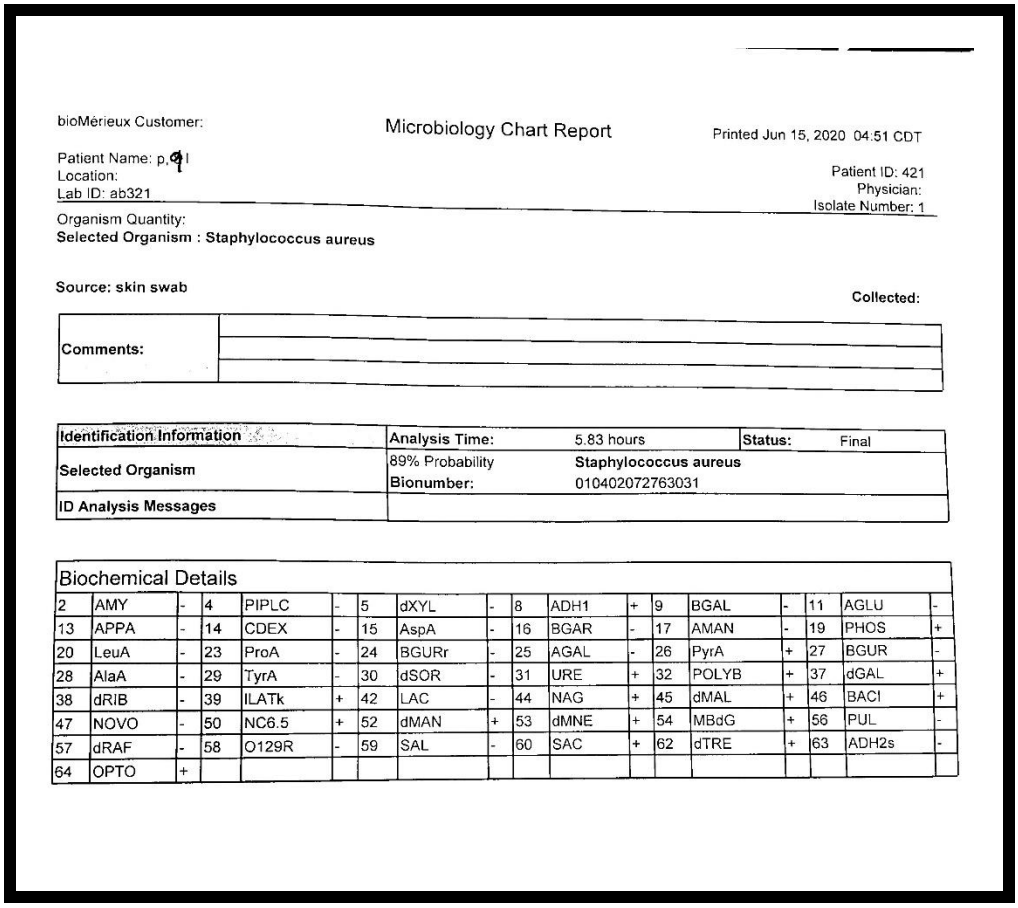
Comments:	

Identification Information	**Analysis Time:**	5.87 hours	**Status:**	Final
Selected Organism	97% Probability **Bionumber:**	**Kocuria rhizophila** 010002100000001		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	+	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	-	37	dGAL	-
38	dRIB	-	39	ILATk	-	42	LAC	-	44	NAG	-	45	dMAL	-	46	BACI	-
47	NOVO	-	50	NC6.5	-	52	dMAN	-	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	-	62	dTRE	-	63	ADH2s	-
64	OPTO	+															

Appendix



bioMérieux Customer:

Microbiology Chart Report

Printed Jun 15, 2020 04:51 CDT

Patient Name: p,q l
Location:
Lab ID: ab321

Patient ID: 421
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus

Source: skin swab

Collected:

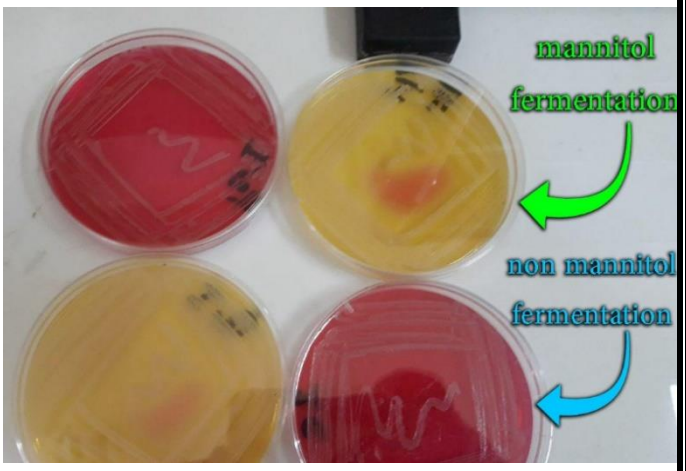
Comments:	

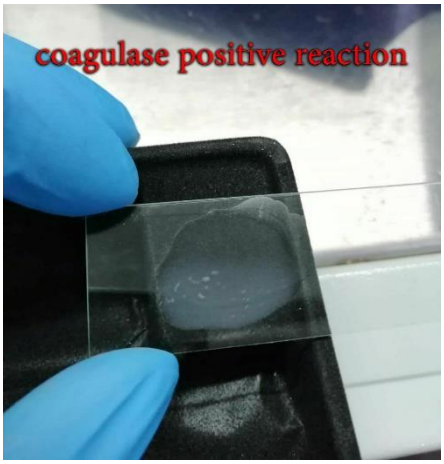
Identification Information	**Analysis Time:**	5.83 hours	**Status:**	Final
Selected Organism	89% Probability **Bionumber:**	**Staphylococcus aureus** 010402072763031		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	+	37	dGAL	+
38	dRIB	-	39	ILATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Appendix(10): biochemical test for isolated bacteria





الخلاصة

التهاب الجلد الاستشرائي و هو مرض متعدد الاوجه ينتج من علاقة معقدة بين عوامل بيئية ووراثية . فضلا عن الدور الذي تلعبه عدوى سطح الجلد والاستجابة المناعية بزيادة حدة هذا المرض.
أجريت الدراسة الحالية لتقييم الارتباط بين مستوى الانترليوكينات والغلوبيولين المناعيIgE في المصل لبعض المتغيرات المناعية مثل (انترلوكين 5 (IL-5) ، انترلوكين 13 (IL-13) و الغلوبولين المناعي IgE)) مع زيادة تردد العدوى البكتيرية في الجلد وزيادة شدة الاعراض الالتهابية لدى المرضى المصابين بذلك المرض .

حيث تم جمع عينات الدم ومسحات الجلد من 80 شخص في هذه الدراسة المسجلين ، من بينهم 50 مريضاً (20 ذكور و 30 إناث) لديهم تاريخ مرضي للإصابة بمرض التهاب الجلد الاستشرائي الذي تم تشخيصه حسب الأعراض السريرية ومن قبل أطباء الاختصاص. بالإضافة الى30 شخص يتمتعون بصحة جيدة كمجموعة سيطرة, ذلك خلال المدة من كانون الأول 2019 إلى تموز 2020 ، حيث تم تنفيذ إجراءات الدراسة في مختبرات مستشفى الحسين التعليمي في كربلاء و في مختبرات كلية العلوم/جامعة كربلاء ، عند التشخيص حسب العمر والجنس. أظهرت البيانات التي تم الحصول عليها من الدراسة الحالية أن نسبة الإصابة بمرض التهاب الجلد الاستشرائي عند الإناث كانت أعلى (44%) من الذكور (28%) ، بالإضافة إلى أن الفئة العمرية 5-15 كانت الأعلى نسبة إصابة بمرض التهاب الجلد الاستشرائي(44%) مقارنة بغيرها من الفئات العمرية.

كما تم زراعة 130عينة مأخوذة من الجلد (مسحة) من 50 مريض بمرض التهاب الجلد الاستشرائي (50 من منطقة الاصابة بالمرض و50 من المنطقة المحيطة بالإصابة) و30 مسحة من مجموعة السيطرة , وتم تشخيصها بالفحوصات الكيميائية, تقنية الفايتك و باستخدام تقنية الPCR حيث كانت نسبة البكتريا الموجبة لصبغة غرام 31 عزلة (91%) متمثلة ببكتريا *Staphylococcus aureus* (70.5%) حيث انها البكتريا الاكثر شيوعا تليها Bacillus spp (8.8%) ومن ثم *Staphylococcus. epidermidis* (2.9%) , *Kokuria rhizophila*(5.9%), *Staphylococcus heamolyticus*(2.9%), اعلى من نسبة الاصابة بالبكتريا السالبة لصبغة غرام متمثلة ببكتريا *Pseudomonas stutzeri* 3 عزلات (8.8%) في المرضى المصابين بالتهاب الجلد الاستشرائي.

بالإضافة إلى البكتيريا الموجبة جرام التي تمثلت بال *Staphylococcus. aureus* و *Staphylococcus .epidermidis* و *Staphylococcus. heamolyticus* تم عزلها من المنطقة المحيطة بمنطقة الإصابة بمرض التهاب الجلد الاستشرائي بنسبة عالية مقارنة بالبكتيريا سالبة الجرام ، وهي عزلة واحدة (3%) ممثلة بـ (*Pseudomonas stutzer*i). علاوة على ذلك ، فإن البكتريا المعزولة من جلد مجموعة السيطرة تمثلت بنسبة منخفضة 4عزلات من *Staphylococcus. aureu*s (20 %) مع نسبة عالية من عزلات الفلورا الطبيعية 16 عزلة (80%) *Staphylococcus. epidermidis*.

كما تم جمع عينات الدم من المرضى لتحديد مستوى المصل من IgE و IL-5 و IL-13 في جميع المجموعات المدروسة ، وأظهرت النتائج زيادة معنوية في مجموعات المرضى (378.2) (13.3) (23.6) على التوالي مقارنة بمجموعة السيطرة. المجموعة التي تصل إلى (65.1) (2.2) (2.06). بالإضافة إلى ذلك ، أظهرت نتائج تعداد كرات الدم البيضاء الكلية زيادة معنوية ($P < 0.05$) في المرضى المصابين بمرض التهاب الجلد الاستشرائي في الإناث (8.48) والذكور (7.80) مقارنة بالذكور (6.86) والإناث (7.10) بالمقارنة مع مجموعة السيطرة. ومن التباين المعنوي في تعداد خلايا الدم البيضاء ، أظهر متوسط الخلايا العدلة (neutrophils) زيادة معنوية في الإناث (51.72) مقارنة بالذكور (46.21) ، بالإضافة إلى زيادة الخلايا اللمفاوية (lymphocytes) لدى الذكور (37.42) مقارنة بالإناث (36.02) ولكنها اظهرت زيادة غير معنوية في الذكور مقارنة بمجموعة السيطرة. أظهرت الخلايا الحمضة (Eosinophils) والقعدة(Basophils) زيادة معنوية ($P < 0.05$) في كلا الجنسين مقارنة بالمجموعة السيطرة.

يمكن الاستنتاج من هذه النتائج أن هناك علاقة بين تباين بعض المعايير المناعية لدى مرضى التهاب الجلد الاستشرائي مع المرض وتطور العدوى البكتيرية.

جمـــــهوريـــة العـــراق
وزارة التـعليـــــم العالـــي
والبحـــــث العـــلمـــــي
جامعـة كربلاء/كليـة العلـوم
قســـــم علـــــوم الحيـــاة

المحددات البكترولوجية والمناعية لمرضى التهاب الجلد الاستشرائي في محافظة كربلاء المقدسة

رسالة

مقدمة الى مجلس كلية العلوم ـ جامعة كربلاء

وهي جزء من متطلبات نيل شهادة الماجستير في علوم الحياة

من قبل الطالبة

زهراء هادي عبد الامير الوزني
(بكالوريوس علوم/ علوم الحياة-2017)

بأشراف

أ.م.د. علي عطيه عبد الحسناوي أ.د. وفاء صادق محسن الوزني

شعبان2 144هـ نيسان2021م