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Role of Bacterial Infection in Autoimmune Disease

A Thesis

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بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

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صدق اللهُ لعَلِيُ العَظيم

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Supervisors Certification

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Dedication

To the teacher of humanity and the savior of humanity (Prophet Muhammad peace be upon him).

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To all my family and friends

I dedicate my condescending message

Abstract

Systematic autoimmune lupus erythematosus (SLE) is disease that impacts several tissue and organs leads to damage of these tissues.The present study has been conducted in Karbala province. A total of (60) blood and urine samples, (40) from patients women and (20) from control women were taken from women aged (20-59) years. This patient`s group divided into two groups, women suffer from urinary tract infection and the second group without infection. To determine the effect of bacterial infection on the level of (CD4+Tcell, CD19+B cell, HLA-DR and HLA-DQ) in blood using flowcytometry technique and identify the level of(interleukine-10 and interleukine-23) in serum using ELISA technique, molecular method using polymerase chain reaction technique to determine *csgA* presence in bacterial samples have been used.

The present study has recorded (15) infection with different types of bacteria that infect urinary tract in patients with SLE, the identification of bacterial using biochemical test and confirmed using viteck system.

The results showed significant increase in the level of CD4+Tcells in patients that have systematic lupus erythematosus ($P \leq 0.0001$) and compared to in SLE patients with infection than without getting an infection as well as control, the results also showed significant increase in the level of CD19+B cells in SLE patients compared to control, and significant increase in the level of CD19+B cells in patients that have infection (*csgA* positive) ($P \leq 0.05$) relative to patients without infection.

Furthermore, the result showed significant decrease in the level of HLA-DR in patients compared to control ($P \leq 0.01$) and also in patients with infection compared to control ($P \leq 0.05$). While the level of HLA-DQ showed decrease in patients compared to control and increase in patient with infection compared to patients without infection but the result was not significant.

Finally the results of the study demonstrated that there was significant increase in concentration of proinflammatory cytokine IL-23 in Compared between patients to control($P \leq 0.001$) And also significant increase in patients with infection relative to control($P \leq 0.0001$), while results of the concentration of anti-inflammatory cytokine IL-10 showed no differences in Compared with monitoring, all groups of patients.

The current study concluded that the bacterial infections in SLE patients that cause UTI especially bacteria that express csgA gene activate the adaptive immune response especially T and B cells.

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List of abbreviations

Anti-ds DNA	Anti-double strand deoxy nucleotide
APCs	Antigen presenting cells
ANA	Anti-nuclear antibody
CD	Cluster of differentiation
Csg A	Curl specific gene A
DC	Dendritic cells
DAMP	DNA-association molecular pattern
ELISA	Enzyme linked immune sorbent assay
Et al	Et alil
EDTA	Ethylene demine Tetra acetic acid
ECM	Extracellular matrix
HLA	Human leukocyte antigen
IL	Interleukine
KDA	Kilo Dalton
MHC	Major histocompatibility complex
NETS	Neutrophil extra cellular traps
PAMP	Pathogen-associated molecular pattern
PBS	Phosphate Buffer Solution
PDC	Plasma cytoid- dendritic cell
PCR	Polymerase chain reaction
Treg	Regulatory Tcell
SLE	Systematic lupus erythematosus
Th	T helper
Th1	T helper type 1
Th2	T helper type 2
TLRS	Toll-like receptor
TBE	Tris Borate EDTA
TNF	Tumor necrosis factor
UV	Ultra violet
USA	United State Of America
UTI	Urinary tract infection

CHAPTER ONE

1.1. Introduction

Autoimmune disease (AID) A category of disorders which in inflammatory respone are the main cause Via the immune system that targets the tissues of the body itself. Both the Inherent human genetics vulnerability and environmental Factors have a crucial role to play in deciding the potential risk of risk of autoimmune disease formation. Infection is The ecological factor which is thought to play a major role in the autoimmunity formation(Nielsen et al., 2016).

Systematic Lupus erythematosus (SLE) is a group of autoimmune diseases which are characterized by inflammation of the vascular and connective tissue. All organs may be impacted by lupus, but kidneys, heart, nervous system and joints are the most systemically affected organs and skin is affected in all groups. Systemic lupus erythematosus is more common in the adult population of women (female / male: 9/1), but it is not common in children and about 20 % of patients are children under the age of 15 (Tahernia *et al*., 2016).

The immune system provides vital protection against pathogenic micro-organisms, but it can also damage self-tissue.CD4+T cells organize various immune responses to different disease causing pathogens. Activated CD4+T cells are classified into many subsets of effector cells with different functions.These types of cells include type I (Th1), Th2, Th1 7, T follicular helper (Tfh), and T (Tregs) regulatory cells. Th1 and Th17 cells may cause autoimmune disease, but the Tregs suppress it (Lee, 2018).

In humans two different subsets of Th17 cells were identified. The non-pathogenic subtypes whose main function is to protect against pathogens in mucous membranes and pathogenic Th17, which are elevated

in peripheral lupus blood. In SLE, disease activity may be associated with an imbalance between these subsets, with a prevalence of pathogenic Th17 and lower concentrations of those cells dedicated to protecting against infection (Torres-Ruize *et al*., 2019).

The excessive activation of the complementary pathway is one of the key reasons for tissue insults and final organ harm in autoimmune disease (Ballanti *et al*., 2013; Lintner *et al*., 2016). Complement is a system of soluble blood proteins secreted mainly from liver hepatocytes that operate in the opsonization of bacteria and viruses. the direct killing of bacterial cells and the clearance of immune complexes by the creation of a complex of membrane attack (MAC) (Biesecker *et al*., 2018).

The diagnosis of autoimmune disease (AID) depends on the history of the patient, physical examination and radiological and laboratory examination, different techniques may be used to detect autoantibodies in a patient with suspected autoimmune disease (Rondeel *et al*., 1999).

Infectious agents, including viruses, bacteria, parasites and fungi, are a central factor in autoimmune induction. Many pathogens in genetically susceptible individuals are known to lead to abnormal immune responses through molecular mimicry, epitope spread, by stander activation or other mechanisms (Espositeo *et al*., 2014).

Several evidence, such as the seasonal onset of disease, the incidence of disease after certain infections, and the isolation of viral genomic DNA/RNA from infected patient tissues once the disease has begun, have supported the pivotal role of infection in the induction of these disorders (Zampier *et al*., 2006).

Autoimmune disorders and pathogens have multidirectional and multifaceted interactions. However, This has been proposed this infection can not just that cause Autoimmune disorders, or precipitate them, But it can also be defend against autoimmunity or even with the abrogate an existing process of autoimmune based on the relationship among microorganisms and the host.We need to have, therefore, Look not just at the microorganisms as they are sources of infection but also as possible the agents that can the immune system modulates (Sfriso *et al*., 2010).

Curli, a type of amyloid-like fimbriae, are considered a virulence factor because of its role in bladder cell adherence and the formation of biofilm (Hollenbeck *et al*., 2018).

The host immune system recognizes curli amyloid fibers, resulting in a signaling cascade that alters bacterial-host interaction. Various immune cells recognize curli fibers through the hetero complex TLR2/TLR1 / CD14 (Rapsinski *et al*., 2013). Curli fibrils are also considered major virulence factors suggested to promote the spread of host bacteria. Curli is recognized by Toll-like receptors, leading to innate immune system activation.Thus, curli is considered pathogen-associated molecular patterns (PAMPs) (Dueholm *et al*., 2012).

CsgA is the curli fiber's major structural subunit, forming several hundred unit long homopolymers both *in vivo* and *in vitro*. CsgA's amino acid sequence includes three well-described domains: a signal peptide, an N-terminal 22-amino acid targeting sequence (N22), and the core domain of C terminal amyloid. Curli are noncovalent heteropolymeric filaments of subunits CsgA and CsgB, present in *in vivo* wild-type fibers at ratios of approximately 20:1 CsgA: CsgB. Curli belong to an ordered class of stable protein aggregates known as amyloids (Van Gerven *et al*., 2015).

One of the most well-researched bacterial amyloids is Curli.; Curli is the one produced By the members of the family Enterobacteriaceae, including Salmonella entericaserovar Typhimurium and E.coli (Tursi *et al.*, 2017).

1.2. The aim of the study

The current study aims to investigate the role of some virulence factors founded in bacteria upon immunological markers and evaluate the level and effect of each one in Systemic lupus erythematosus patients, this will achieve by:

1. Detection of virulence factor, and
2. Determine the level of some human leukocyte antigen HLA-DQ, HLA-DR, inerlukin-10 and interlukin-23, CD4 and CD19.

CHAPTER TWO

2. Literature review

2.1. Immune response to bacterial infection

2.1.1. Innate immune response

The innate immune system is the first responder to invade microorgaisms which are the main components of the innate immune system: Natural defenses (skin, mucous membranes, etc), unspecific cells (phagocytes, natural killer cells, etc), and non-specific molecules (complements, interferons, etc).In addition, many factors affect how the immune system responds to microorganisms such as age, general health, nutrition, and genetic makeup of any human host (Reygaert, 2014). Innate immune response may be mediated by stimulation intrinsic or extrinsic to it. Extrinsic stimulation is mediated by receptors of host cells (cells shouldn't have infected). Intrinsic activation in infected host cells is mediated by intracellular signals (Iwaski, 2010). One feature of the innate immune system is the detection by toll like receptors (TLRs) of pathogen-specific components. Toll-like receptors are a family of transmembrane proteins which are sensors to pathogen-associated molecular patterns (PAMPs) (Gluba *et al*., 2010). Under normal circumstances, the immune system protects against infectious organisms, requiring it to distinguish foreign agents from healthy tissues of the host, including viruses, bacteria, fungi and parasites. However, failure to distinguish between foreign and host can lead to the development of autoimmune diseases, including organ-specific diseases with limited tissue involvement, such as multiple sclerosis and type I diabetes, or more systemic involvement, such as systemic lupus erythematous (SLE). Autoimmune disease

components of both the innate and the adaptive immune response are needed for most idiopathic autoimmune disease (Mills, 2011; Thofilopoulos *et al*., 2010). The innate immune system provides wide protection against pathogens and most multicellular organisms rely on it to fight against microbial infections. The innate immunity mechanism is the secretion of antibacterial substances of wide spectrum, such as small cationic polypeptides (3–5 kDa) called defensins (Ganz, 2003). All of the defenses identified to date are capable of killing and/or inactivating a spectrum of bacteria, fungi and some enveloped viruses (Yang *et al*., 2002).This ability to kill microorganisms is attributed to their ability to disrupt the integrity and function of membranes (Lehere, 2002). Inflammatory and infectious processes include neutrophils, which are critical components of the innate immune system. Improper neutrophil activation can release protease, tissue damage factors, and reactive oxygen species, resulting in SLE tissue damage (Smith *et al*., 2015). Neutrophils with a new structure were found to be involved in autoimmune disease: extracellular traps neutrophils (NETs). NETs are fibrous networks of nuclear and granule components protruding from the neutrophil membrane activated. Studies have shown that vasculitis and SLE autoantibodies are NET components (Kufman, 2017; Czaikosk *et al*., 2016).The innate immune system has one specific phagocyte, the dendritic cells (DCs),which function as qualified antigen presenting cells and play a very important role in connecting innate immunity with acquired immunity (Steinman *et al*., 2003). DCs can either induce immunity, or tolerance. It has been suggested that the dendritic cells contribute to SLE pathogenesis.

This is demonstrated by the findings that high IFN-α level contributes to the development of SLE (Ronnblom *et al*., 2003), and the major cells involved in the production of IFN-α are activated pDCs (Barrat *et al*., 2005).

2.1.2. Adaptive Immunity

Adaptive immune responses are superior to innate immunity, as they provide pathogenic specificity and immune memory, effectively avoiding potential host reinfections with the same pathogens (Pancer and Cooper, 2006). Lymphocytes are the main agents of adaptive immunity. There are two major lymphocyte populations: T cells responsible for cell-mediated immunity and immune control (Mchugh and Shevach, 2002) and B cells responsible for humoral immunity (Youinou , 2007). CD4+T cells in lupus are critical drivers of the B-cell-dependent autoantibody response by supplying costimulatory signals and cytokines (Herman *et al*., 2000). CD4+T cells that orchestrate immune responses may be divided into Th1, Th2, and Th17 cells based on the cytokines that they primarily generate (for example, IFN-γ, IL-4, and IL-17, respectively (Reiner, 2007). Helper T cell (Th cell) differentiation is critically dependent on the local cytokine environment and co-stimulation by antigen-presenting cells (APCs) (Reiner, 2007).Th1 cells are characterized by interferon-γ production, which is essential to the defense against intracellular pathogens. Th2 cells are characterized by IL-4 developments, and are critical in the protection of the host against parasite infections. A new subset of Th cells called Th17 cells has been identified and these cells are characterized by IL-17 developments (Park *et al*., 2005). Th1 dominated the Th1/Th2 balance in the chronic course of SLE, particularly in patients with lupus nephritis (Dolff *et al*., 2011). Th17 cells in the presence of TGF-β and IL-6

differentiate from naive CD4+T cells by stimulation with antigen. IL-17 and IL-22 secret cells Th17 (Agmon *et al*., 2012).Th17 is responsible for mounting the immune response against fungi and extracellular bacteria.

They also participate in the creation of autoimmune diseases (Annunziato *et al*., 2007). Studies in mice and humans indicated that Th17 cells could be important tissue damage mediators in immune-mediated inflammatory diseases such as SLE (Scheinecker *et al*., 2010; Alunno *et al*., 2012). T helper 17 (Th17) cells are distinguished from native CD4+T cells and are characterized by IL-17 production and have been included in the list of autoimmune diseases such as SLE as they are responsible for the inflammatory response in these diseases (Park *et al*., 2005). Most of the studies on Th17 and Treg that studied their relationship to (SLE) disease showed that Th17 elevation and Treg reduction were considered to be the major factor in increasing the production of autoantibodies and tissue damage in SLE patients (Yang *et al*., 2009).The bulk of T-lymphocytes contain CD4+T cells along with CD8+T cells. Upon activation and differentiation into distinct effector subtypes CD4+T cells play an important role in the regulation of immune response through secretion of different cytokines. CD4+T cells perform multiple functions, through cell activation of the innate immune system, B-lymphocytes, cytotoxic T cells and non-immune cells play a critical role in the suppression of immune reaction (Luckheeram *et al*., 2012). CD19 is a B cell-specific member of the Ig superfamily expressed by early pre-B cells from the time of heavy chain rearrangement until plasma cell differentiation. The cell surface density of CD19 is tightly regulated during B cell differentiation particularly in mice (Sato, 2000).

2.2. Systematic Lupus Erythematosus (SLE)

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by autoantibodies and immune complex deposition that affects a wide variety of organs. It is thought that genetic factors, environmental factors and hormonal factors contribute to SLE. Innate and adaptive immune responses to self-antigen trigger the formation of auto-antibodies and the deposition of immune complexes in tissues leads to complement activation, aggregation of neutrophils and monocytes, and self-reactive lymphocytes (Tskos *et al*., 2016). It often affects young women during periods of childbearing; however, it can also occur in children, the elderly and males at any age (Hwang *et al*., 2015). Immunologically, the disease is characterized by polyclonal B cell activation and overproduction of anti-nuclear autoantibodies, resulting in a deposition of the immune complex that causes inflammation and tissue damage (Castro *et al*., 2008). Another immunological feature of SLE patients is an abnormality in the T-cell response manifested by an imbalance in cytokine production which contributes to tissue damage (Marks *et al*., 2012).

It is a type III hypersensitivity reaction caused by complex formation of antibody-immune. The course of the disease is unpredictable, with remissions alternating with periods of illness (called flares) (Rahman and Isenberg, 2008).The first study relating to SLE in Iraq was in 1971. There was a 10-year follow-up on 36 SLE cases in which clinical, laboratory and pathological studies were conducted (AL-Damlujii *et al*., 1971).

In Iraq, the SLE was considered the third most common inflammatory rheumatic disease after rheumatoid arthritis and rheumatic fever with 53/100,000 incidence of Iraqi population (Al-Rawi *et al*., 1983).

2.2.1. Epidemiology of Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a widespread illness that affects several organs. Epidemiological studies suggest that SLE occurs differently across countries and even among different areas of the same country (Hqokinson, 1992).There are also variations between population groups of the same race living in different parts of the world, indicating that environmental factors contribute to the development of SLE. The incidence and prevalence of the disease also appear to be influenced by ethnic and geographical factors (Manzi, 2001). Long-term survival is usually less likely in Asia than in America and Europe (Tikly and Navarra, 2008; Alamanos *et al*., 2003). SLE's global incidence ranges from about 1 to 15 per 100,000 person per year. The SLE prevalence rates range from roughly 15 to 150 per 100,000 (Arnaud and Vollenhoven, 2018).

2.2.2. Disease activity

Three main patterns of disease activity were identified, including a remitting-relapsing course of disease characterized by flares and remission periods, chronically active disease and long quiescence (Arnaud and Vollenhoven, 2018).

2.2.3. Systematic Lupus Erythematosus Diagnosis

There is no specific diagnostic marker on the disease; rather, it is characterized by a combination of clinical and laboratory criteria. Precise diagnosis of systemic lupus erythematosus is important, because treatment can reduce morbidity and mortality, particularly from lupus nephritis. For children and adults the clinical symptoms of systemic lupus erythematosus are essentially the same. In two retrospective studies of children with the disorder fever, rash, arthritis, alopecia and renal involvement were the most common symptoms (Gill *et al*., 2003).

2.2.4. Diagnostic marker

-ANA

Anti-nuclear antibody (ANA) presence of antinuclear antibody (ANA) at a titer ≥1/80 is the most sensitive diagnostic criteria for SLE, as it is observed in virtually all patients with the disease. ANA can be detected in the blood several years before the diagnosis (Arnaud and Vollenhoven, 2018).

-Anti-dsDNA

A classic autoantibody that characterizes SLE is the anti-double stranded DNA (dsDNA). Anti-ds DNA antibodies to DNA are specific to disease and are closely related to outbreaks of renal activity and disease flares. Anti-ds DNA antibodies are also through to be directly involved in SLE pathogenesis by forming immune complex that cause organ damage, such as lupus nephritis. Many SLE patients do, however, lose serum anti-ds DNA reactivity during their disease course (Min *et al*., 2002).

Due to their high diagnostic ability, anti-ds DNA antibodies are of particular interest to SLE, with approximately 70-80% of patients being positive for such antibodies and titers typically associated with disease activity (Mustelin *et al*., 2019).

2.2.5. Disease Manifestation

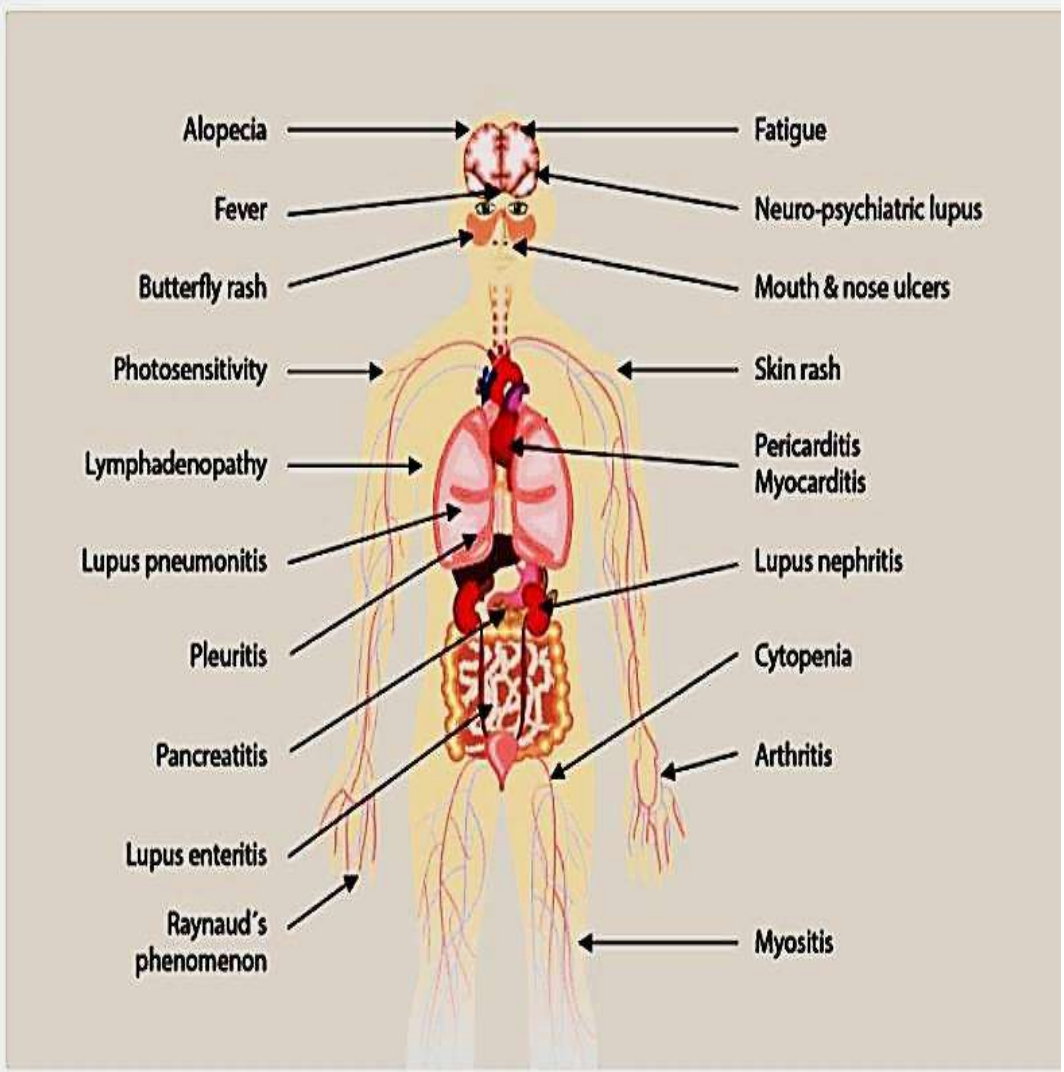


Figure (1-1) Anatomical depiction of disease manifestation of systematic lupus erythematosus (Arnaud and Vollenhoven, 2018).

2.3. Classification Criteria

The classification criteria for SLE were cited in 1971, revised in 1982, and revised again in 1997 (Hochberg, 1997).These criteria are based on the common signs and symptoms of SLE. Lupus erythematosus is diagnosed when any four or more criteria are present (Griffiths, 2005; Smith and Gordon, 2010).

Table (2-1) : criteria were used for the diagnosis of lupus, as defined by the American college of rheumatology

Malar rash	The classic raised, red rash that looks like butterfly over the nose and cheek
Discoid rash	Hard, raised areas of scaly skin
Photosensitivity	Reaction to sunlight, resulting in the development of or increase skin rash
Oral ulcers	Sores in the mouth , usually painless
Arthritis	Red, tender and swollen one or more joints. The cartilage which is the protective tissue surrounding the bone, remains intact
Serositis	Inflammation of pleurae or pericardium, respectively; may cause pain when breathing deeply
Neurologic Disorder	headaches, seizures, amnesia, decreased ability to concentrate
Kidney disorder	Excessive protein in the urine (greater than 0.5 gm/day or 3+ on test sticks) and/or cellular casts
Hematology disorder	Hemolytic anemia or leucopenia (<4,000cu/ml)، lymphopenia (<1,500 cu/ml) or thrombocytopenia (<100,000platelets cu/ml). The leucopenia and lymphopenia must be detected on two or more occasions. The thrombocytopenia must be detected in the absence of drug known to induce it
Immunologic Disorder	The presence of antibodies such as anti-DNA, anti-Sm, or antiphospholipid
Positive ANA	increased susceptibility to infection , involvement of various organ systems by inflammation

2.4. Bacterial Infection in Lupus Patient

Within SLE, bacterial infections commonly occur and affect various organs, including the respiratory tract, urinary tract, and skin (Goldblatt *et al.*, 2009). Infections are the leading cause of SLE patients ' morbidity and mortality, accounting for up to 55 percent of SLE deaths. Bacterial infections can expose the immune system through two main processes to

nuclear material and nucleic acids in particular-induction of release of host nuclear autoAgs by causing cell death, either directly or as a result of the immune responds to pathogen, and bacterial DNA release due to bacterial death or successful extrusion. Endogenous DNA such as mitochondrial DNA can function as DAMP and be recognized by B cells that are autoreactive. Similarly, by functioning as PAMP and activating autoreactive B cells through molecular mimicry, bacterial DNA, probably associated with other bacterial molecules, will induce autoimmunity (Qiu *et al*., 2019). Diminished phagocytosis, reduced interleukin 8 (IL-8) and IL-12 development by polymorphic nuclear cells, complement deficiency and defective chemotaxis, membrane recognition and microorganism attachment are known to predispose SLE patients to infection (Cuchacovich *et al*., 2009). Bacterial lipopolysaccharides, or immune complexes containing nucleic acid, engage and weaken the immune system (Francis *et al*., 2010). Bacterial products which are called pathogen-associated molecular patterns bind on APCs, B cells and T cells to TLRs or other receptors. Our interactions trigger immune cells to produce proinflammatory cytokines which cause activation of innate immunity (Deng *et al*., 2008).

2.5. Interleukin10

Interleukin 10 (IL-10) is a key immunoregulatory cytokine that almost all leucocytes can produce it, innate immune cells such as monocytes, macrophages, DCs, mast cells, natural killer cells, eosinophils, neutrophils and adaptive immune cells such as Th1, Th2, Treg, Tr1, Th3, CD8+T, and B cells (Rhodes *et al*., 2008; Yanaba *et al.*,2009). Antigen presenting cells and lymphocytes are the main targets of IL-10 on immune cells. On the other hand, IL-10 inhibited the antigen-presenting ability of

monocytes and macrophages by down-regulating the MHC class II cell surface, costimulatory molecules such as CD86, and adhesion molecules such as CD58 (Su *et al*., 2012). IL-10 stimulated the formation of type 2 cytokine pattern by inhibiting T lymphocyte growth in IFN-γ (Romagnani, 1995).The proliferation of CD4+T cells and the development of cytokines such as IL-2, IFN-γ, IL-4, IL-5 and TNF-α is specifically inhibited (Joos *et al*., 2000). In UTIs, IL-10 is primarily produced early by monocytes, probably this production is induced by LPS reconnaissance.

It has been characterized as a regulator of the innate immune response, having multiple upregulatory and downregulatory immune functions. In UTIs, the main function may be to protect the host from exaggerated immune responses which produce inflammation and tissue injury (Mege *et al*., 2006; Duell *et al*., 2012).

2.6. Interleukin23

Interleukin 23 (IL-23) is a heterodimeric cytokine consisting of subunits IL-12 p40 and IL-23 p19 and belongs to the cytokine family IL-12. Antigen-presenting cells as well as T cells and endothelial cells make up the p19 subunit of IL-23. The p40 subunit is essentially limited to antigen-presenting cells such as monocytes, macrophages, and dendritic cells (Langrish *et al*., 2004; Kastelein *et al*., 2007). Macrophages and dendritic cells (DCs) secrete IL-23 in response to inflammatory cytokines and microbial products (Langrish *et al*., 2004). IL-23 plays a crucial role in the production of pathogenic Th17 cells generating cytokine IL-17 (Cornelissen *et al*., 2009). IL-23's pro-inflammatory activities is partially due to its ability to support the development of a novel subset of CD4+ inflammatory T cells known as Th17 cells (Bettelli and Kuchroo, 2005;

Harrington *et al*., 2005). IL-23 also has active effects of monocytes and macrophages on the cells of the innate immune system, inducing the development of inflammatory cytokines such as IL-1, IL-6 and TNF-α (Mckenzie *et al*., 2006; Puccetti *et al*., 2002).

IL-23 has been reported to enhance the peripheral mononuclear blood cell (PBMC) IL-17 secretion from healthy subjects (Gocke *et al*., 2007). Clinical studies of several immune-mediated disorders, including Crohn's disease, rheumatoid arthritis, and multiple sclerosis, find IL-23 to be overexpressed in humans (Langrish *et al*., 2005).Given that cytokine-mediated immunity plays a crucial role in SLE pathogenesis, a possible role of IL-23 is strongly suspected in SLE pathogenesis (Leng *et al*., 2010).

2.7. Biofilm Forming Bacteria in Autoimmune Disease

Biofilms are bacterial communities embedded in the Extra Cellular Matrix (ECM), which defends bacteria against environmental stresses like antibiotics (López *et al*., 2010). Biofilms are formed on various biotic and abiotic surfaces including the mucous surfaces of the human body and medical devices occupied, to defend themselves from environmental or immune stress (Macfarlane *et al*., 2011; Jamal *et al*., 2018). The composition of the biofilm and the design of the biofilm depend on both the bacteria in the biofilm and the setting in which it is located (Flemming *et al*., 2010). Curli developed by enteric Gram-negative bacteria that commonly cause infections in SLE patients, including *Escherichia coli* and *Salmonella spp*, is the best studied bacterial amyloid (Tursi *et al*., 2018). Defines enteric biofilms as curli-producing bacteria, excluding non-curli-producing bacteria such as the species *Klebsiella*, *Shigella* and entero invasive *E. coli* (Zogaj *et al*., 2003; Sakellaris *et al*., 2000). Curli amyloid fibers are the major protein component of the extracellular matrix (ECM)

needed for biofilm formation of floating pellicles. The central curli subunit protein, CsgA, is transported by the corresponding CSGBAC and CSGDEFG operons to the cell surface and polymerized into an amyloid fiber (Evans and Chapman, 2014). A side from their role in the formation of biofilms, curli fibrils contribute to bacterial pathogenesis during various infections, including infections of the urinary tract and sepsis (Kai-Larsen *et al*., 2010).

Amyloid fibrils are not only formed by Enterobacteriaceae members, but are also commonly found in biofilm material from Firmicutes, Bacteroides and Actinobacteria phyla bacteria (Larsen *et al*., 2007; Jordal *et al*., 2009).These are also highly proinflammatory to induce autoimmune responses and can neutralize human cathelicidin (LL-37), a soluble antimicrobial peptide that defends against UTI (Nhu *et al*., 2018). Over the past decade the innate immune response to curli has been thoroughly researched. Curli binds and activates Toll-like receptor 2 (TLR2), which results in the development of proinflammatory cytokines and chemokines such as interleukin 8 (IL-8), IL-6 and alpha tumor necrosis factor (TNF-α) and nitric oxide (Tukel *et al*., 2009; Bian *et al*., 2001). TLR9 is known to recognize bacterial DNA by binding to unmethylated cytosine-guanine (CpG) dinucleotides (Hemmi *et al*., 2003). Activation of TLR9 results in the development of type I IFNs, a family of cytokines that are essential for both bacterial and viral infections that cause pleiotropic immune system activation (Kawai *et al*., 2004). Enteric bacteria biofilms have recently been associated with the development of an autoimmune human disease, systemic lupus erythematosus (SLE) (Gallo *et al*., 2015). While various components of the enteric biofilm can be recognized as PAMPs by the innate immune system, some components aid in immune evasion and thus

promote bacterial virulence. Although curli is recognized as a PAMP, curli can protect *E. coli* against complement-mediated phagocytosis (Biesecker *et al*., 2018).

2.8. Human leucocyte antigen (HLA)

The major histocompatibility complex (MHC) is a large gene complex with an integral role in the immune system that is found in all jawed vertebrate classes. Cell surface glycoproteins that bind intracellular and extracellular peptides, respectively, are the antigen-presenting molecules encoded by the MHC class I and class II genes. The human MHC resides on chromosome 6 and contains over 200 genes. Human glycoproteins encoded with MHC are known as human leukocyte antigen (HLA) and are specialized in presenting short peptides to T cells and play a key role in the body's immune defense (Mosaad, 2015). The HLA can be divided into three sections, labeled I, II, and III stages. Classical classes I and II loci encode proteins of the human leukocyte antigen (HLA) involved in the presentation of antigen to T cells (Morris *et al*., 2012). The main function of HLA class I molecules, expressed in all nucleated cells, is to present non-self-derived antigens from intracellular sources, such as viruses, to CD8+T cells (cytotoxic T cells) which then destroy the antigen-presenting cells (APCs). CD8+T cells interact through their T-cell receptor (TCR) and co-receptor molecule CD8 with cognate peptide-MHC I complexes (Matzaraki *et al*., 2017).

The class II area contains the standard HLA-DP, HLA-DQ and HLA-DR gene loci as well as the non-classic HLA-DO and HLA-DM loci. The classic genes are expressed on the surface of professional APCs, which absorb antigens derived from extracellular sources such as bacteria or food, and present them to CD4+T helper cells. This leads to the secretion of

various small proteins including cytokines that regulate other immune cells such as macrophages or B cells (Matzaraki *et al*., 2017).

The most important function of HLA molecule is in the induction, regulation of immune responses and the selection of the T cell repertoire (Shankarkumer, 2004). HLA-DQ antigen is a molecule of HLA class II encoded by the 2 DQ genes, DQA1 and DQB1. Although the genes DQA1 and DQB1 are both polymorphic, the gene DQB1 is more polymorphic and is the major determinant of the antigen DQ (Rezaei *et al*., 2008).The genes HLA-DRB1 and HLA-DQ have been known to predispose to SLE and lupus nephritis and have been extensively investigated. HLA-DRB1 polymorphism was the most common of all (Shimane *et al*., 2013).

DQ antigens are also involved in identifying specific self-antigens and introducing those antigens into the immune system to develop tolerance from a very young age. After immunity to self-proteins is lost, DQ may become involved in autoimmune disorders. The α and ß subunits are encoded by different genes. The α subunit is encoded by the HLA-DQA1 gene while the HLA- DQB1 gene encodes β. These loci are adjacent to each other on chromosome 6p21.31. A person often produces two subunits of α and β chain that result in isoforms of the DQ four. Different DQ isoforms can bind to different T-cells and present different antigens (Delli *et al*., 2012).

2.9. Urinary Tract Infection

Urinary tract infections (UTIs) are a serious public health problem and are caused by a variety of pathogens but most commonly by *Escherichia coli*, *Klebsiella pneumoniae, Proteus mirabilis*, *Enterococcus faecalis* and *Staphylococcus saprophyticus* (Flores *et al*., 2015).

Urinary tract infections are among the most common bacterial infections affecting 150 million people each year worldwide (Stamm *et al*., 2001). Clinically, uncomplicated or complicated UTIs are graded. Such diseases are usually classified into lower UTIs (cystitis) and upper UTIs (pyelonephritis) (Hooton, 2012), for individuals who are otherwise stable and do not have any anatomical or neurological urinary tract disorders. Complicated UTIs are characterized as UTIs associated with factors affecting the urinary tract or host defense, including urinary obstruction, neurological retention, immune suppression, renal failure, renal transplantation, pregnancy, and the involvement of foreign bodies (Levison and Kaye, 2013).

2.10. Enterobacteraceae

The Enterobacteraceae family is characterized by the following characteristics: gram-negative bacilli (2–4 microns long by 0.4–0.6 microns wide), mobile with peritric ciliature or immobile, growing on common culture media, aerobic — facultative an aerobics, fermenting glucose with or without gas production, nitrates reduction to nitrites, and negative oxidase.

In terms of pathogenesis and ecology, enterobacteria are a very heterogeneous family. However, the species making up this family are either parasites, commensals (*Escherichia coli*, *Proteus mirabilis*, and *Klebsiella spp*.) or even saprophytes (*Serratia* spp., *Enterobacter* spp) (Olson *et al*., 2005).

2.10.1. Enterobacteraceae Virulence Factor

2.10.1.1. Curli specific gene (*CsgA*)

Curli are amyloid fibers which from a key part of the UPEC biofilm matrix (Floyed *et al*., 2015). Curli synthesis is regulated by a type VIII secretion mechanism also called the nucleation precipitation synthesis is regulated by a type secretion mechanism also called the nucleation precipitation pathway and involves the expression of seven genes from two divergent operons (csg BAC and csg DEFG) (Chapman *et al*., 2002) .

The csgBAC operon codes CsgA, the CsgB curli nucleator protein, a nd ClsgC, the Chaperone periplasm (Goyal et *al*., 2014). CsgA is considered the main curli subunit, and CsgA, a 13-kDa protein, is a soluble monomer secreted throughout the cell membrane. CsgA is characterized by five incomplete repeating domains (R) of 19 to 23 amino acids containing retained residues of glutamine and arginine comprising the central amyloidogenic domain of CsgA (Wang *et al*., 2007).

The *csgA* gene product is a soluble, unstructured protein that allows for ass embly of CsgB into *in vivo* fibres. CsgA is secreted as monomer from the cell without csgB, and no fibrils are formed (Shu *et al*., 2012).

2.10.1.2. Fimbriae

Fimbriae (pili) are filiform structures arising from the bacterial surface Fimbriae are present in all genera of Enterobacteriaceae. Their production is influenced by the conditions of growth. Throughout fluid cultures they excel best, and fimbriation is often correlated with film formation; however, they are often well formed throughout ordinary agar media (Elhani, 2012).

2.10.1.3. Antigen –O

The lipopolysaccharide antigen is 100 °C thermostable. There are three parts of this antigen: the lipid layer, the "core" component and the polysaccharide (Dougnon *et al*., 2020).

2.10.1.3. Capsular polysaccharides (K antigen)

Capsular and microcapsular polysaccharides exist in many genera of the Enterobacteriaceae family. Most often, the term capsule is used to denote an external material that covers the entire surface of the bacterium and is so strongly tied to other surface structures that it is not fully solubilized in a fluid medium (Dougnon *et al*., 2020).

2.10.2. Some Member of Enterobacteraceae That Cause UTI

2.10.2.1. *Escherichia coli*

Represents a versatile and complex enterobacterial species that may be subdivided into (i) non-pathogenic, commensal, (ii) pathogenic intestinal and (iii) pathogenic extraintestinal strains; This distinction is based primarily on the presence or absence of DNA regions that are often correlated with some pathotypes. This genetic information has been acquired horizontally in most cases, and belongs to the versatile *E. coli* genome, Including plasmids, bacteriophages and islands in genomics (Dobrindt, 2005). *Escherichia coli (E. coli)* was the most common etiological agent of UTI, responsible for almost 80 percent of community-acquired and 50 percent of hospital-acquired infections (Tajbakhsh *et al*., 2016), and appears to form micro-colonies in the urinary bladder lining of the mucosa known as biofilm.

Such biofilms make the organism more virulent in suppressing the host immune response, and lead to the development of antibacterial drug resistance by enclosing it in an extracellular biochemical matrix (Mittal *et al*., 2015).

2.10.2.2. *Proteus mirabilis*

Proteus mirabilis is a gram-negative bacterium of high motility. This organism infects a very high proportion of patients with problematic urinary tracts, that is, urinary tracts with functional or structural defects or chronic devices. Not only does this bacterium cause cystitis and acute pyelonephritis in these patients, but the production of urinary stones, a hallmark of infection with this organism, further endangers the already complicated urinary tract.

Additional evidence indicates association of this species with rheumatoid arthritis (Pearson *et al*., 2008). Such species demonstrates virulence factors associated with adhesion, motility, immunodeficiency, acquisition of nutrients, damage to the host and development of biofilms. *P. mirabilis* develops biofilms in various ecosystems where those produced in a human host play a key role in system infections (Jacobsen *et al*., 2011).

2.10.2.3. *Klebsiella pneumonia*

Klebsiella pneumonia is a Gram–negative bacterium that belongs to the Enterobacteraceae family and is often present in a number of niches. It is a significant nosocomial pathogen involved in various diseases such as pneumonia infections in the urinary tract, bacteremia and wound infections. *K. pneumonia* produces several structures important for virulence, including pili, which help to initially colonize the host and capsular

polysaccharides which protect the organism from phagocytosis. Complement and inhibit macrophage differentiation (Alcantar *et al*., 2013).

2.10.2.4. *Raoultella ornithinolytica*

Raoultella ornithinolytica is an encapsulated aerobic gram-negative bacillus of the family Enterobacteriaceae. The bacterium, formerly named Klebsiella ornithinolytica, was reclassified as Raoultella based on new genetic approaches. R. Ornithinolytica is known to inhabit aquatic environments and can be found in hospital settings. Ornithinolytica isolates are becoming increasingly relevant in human illness. Several cases of infection with the biliary tract, urinary infection and bacteraemia. Caused by R. Ornithinolytica was described (Seng *et al*., 2016).

2.10.2.5. *Pseudomonas lutellae*

An aerobic, non-spore production, Gram negative and rod-shaped bacterium (0.8-2.5 μm) is an unusual opportunistic pathogen. This bacterium, due to the presence of one or more polar flagellas, is motile. It is non-fermented with lactose and grows well on the MacConkey medium. It has been suggested that the use of steroids and other immunodepressant therapies, the presence of a foreign body and postoperative instability predispose to P. luteola infection (Yousefi *et al*., 2014).

CHAPTER THREE

3. Materials and Methods

3.1. Materials

3.1.1. Devices and Apparatus

Table (3- 1): Devices and apparatus used in this study

Item	Company name	Origin
Autoclave	Genex	USA
Balance	Sartorius	Germany
Centrifuge	Kokvsan	Japan
Cooling centrifuge	Hermle Labnet	Germany
EDTA Tube	Xinel	China
Eliza reader	Biotek	USA
Flowcytometer	Mindry	China
Gel electrophoresis	Cleaver Scientific	UK
Gel tube	Als	China
Hot plate with magnetic	Lab Tech	Korea
Incubator	Memmert	Germany
M icropipette 0. 5-10 µL	Dragonlab	China
Micropipette 100-1000 µL	Humapette	Germany
Microscope	Nikon	China
Power supply	Consort	Belgium
Refrigerator	memmert	Germany
Thermocycler(PCR device)	Multigene	USA
Uv transllluminator	Cleaver scientific	UK
Vitic	Biomerieux	USA
Vortex	Roma MB	Italy
Water bath	Julabo	Germany
Water Distillator	Lab Tech	Korea

3.1.2. Chemicals and Biological Materials

The chemical and biological material used in this study and their origins are given in table (3-2).

Table (3-2): chemicals and biological materials

Item	Company name	Origin
Absolute ethanol	J.T.Baker	Netherland
Agarose	Intron	Korea
Free nuclease water	Bio-lab	India
Gram stain	AFCO	Oman
Primer(csgA)	Primer	Korea
Tris-Borate EDTA buffer	Bio basic	Canada

3.1.3. Media

Media used in this study and their origin are given in table (3-3).

Table (3-3): cultures media

Item	Company	Origin
Blood agar	Biotech	France
Brain heart broth	Humedia	India
MacConky agar	Biotech	France

3.1.3.1. Bromophenol Blue Stock Solution (Bio-Basic/ Canada)

Item	Concentration	Storage
Bromophenol blue	0.1% (w/v)	4ºC

3.1.3.2. Red safe stain (Intoron/Korea)

Item	Concentration	Storage
Red Safe	0.625 µg/ml	4ºC

3.1.4. Interleukin ELISA Kits (Elabscience⁄Usa)

Item	Specification	Storage
Micro ELISA plate	8 wells x12 strips	-20℃,6 months
Reference standard	2 vials	
Concentrated Biotinylated detection Ab (100x)	1vials 120ML	
Concentrated HRP conjugate(100x)	1vials 120ml	- 20 ℃ (protect from light),6 months
Reference Standard&Sample Diluent	Vial 20ml 1	4℃ , 6 months
Biotinylated Detection Ab Diluent	1vial 14ml	
HRP conjugate Diluent	1vial 14ml	
Concentrated wash buffer(25 x)	1vial 30ml	
Substrate reagent	1vial10ml	4℃ (protect from light)
Stop solution	1vial 10ml	4℃
Plate sealer	5pieces	
Manual	1 copy	
Certificate of analysis	1 copy	

3.1.5. Flow Cytometry Kit

Name of kit	Compound	Storage	Manufacturer
Anti-human CD19(APC)	Monoclonal antibody	2-8 ºC	USA
Anti-human CD4(PerCp)	Mono clonal antibody	2-8 ºC	USA
Anti-human HLA-DR (PE)	Mono clonal antibody	2-8 ºC	USA
Anti-human HLA-DQ(FITC)	Mono clonal antibody	2-8 ºC	USA

3.1.6. Primers

Primers used in this study were synthesized by intron (Korea).

The name, sequence and product size are given in table (3- 4).

Table (3-4); the gene name, sequence and product size of primers used in this study.

Gene	Name of primer	Sequence of primer 5 -3	Product size (bp)	Reference
CsgA	Forward	ATCTGACCCAACGTGGCTTCG	178 bp	(Silva *et al*., 2013).
	Reverse	GATGAGCGGTCGCGTTGTTACC		

3.1.7. DNA Extraction Kit

Kit (Sigma⁄Bacterial Genomic DNAGenElute ,USA) composed of the following reagents.

Reagents	Quantity	Storage
Gram positive lysis solution	20 ml	Room temp.
Lysis solution T	20 ml	
Lysis solution c	20 ml	
Wash solution 1	50 ml	
Wash solution concentrate	20 ml	
Elution solution	35 ml	

(10 mM Tris-Hcl, 0.5 mM EDTA, PH 9.0)		
Column preparation solution	60 ml	
Proteinase K	3x10 mg	
RNase A solution	1.7 ml	
GenElute Nucleic Acid Binding columns in tube	each 70	
Collection tubes, 2.o ml capacity	3x70 each	

3.1.8. PCR Master Mix (Bio-lab／England)

Item	Storage
Taq DNA polymerase	-20 ºC
DNTP (mixture)	
Reaction buffer	
Loading dye and stabilizer	

3.1.9. Ladder

DNA Ladder; Sizer -100 DNA Marker (Intron／Korea)

Item	Storage
13 bands (suitable for sizing linear and double-strand DNA fragments ranging from 50-1000 bp).	4 ºC

3.2. Methods

3.2.1. Time and location.

The samples were collected in Karbala (Al-Hussein Educational Hospital) and (Al-Zahra Hospital). During the period from July-2019 to November-2019, province and informative questionnaire was organized to each patient.

3.2.2. Sample collection

3.2.2.1. Blood Samples

1- Five ml of venous blood was taken from each patient by using syringe.

2- Two ml of blood sample was placed in EDTA tube of patients and controls for used in anti CD4, CD19, anti-HLA-DR and DQ diagnosis.

3- Three ml of blood samples was placed in gel tube and left for 20 minutes at room temperature to clot, and then the tube was centrifuged at 3000 rpm for 10 minutes to collect the serum.

4- Serum was stored in the refrigerator (freezing) at -20 until using in ELISA.

3.2.2.2. Urine Samples

Amount of urine was collected from each patient using sterile tube, then the sample culture on blood agar and MacConky agar by streaking using sterile loop and incubate 24h at 37ºC after incubation bacteria was isolate depending on microscopic and culture features then bacterial identification by vitic system .

In this study two types of measurement were taken in consideration, comparing between healthy individual (control) and infected individuals, and also among patients themselves, divided patients into two groups depended on culture result (infected and non-infected).

3.2. 3. Preparation of culture media

All media presented in table (3-3) were prepared according to the manufacturing company instructions. The constituents were dissolved in distilled water. Microwave oven was used to dissolve all constituents completely. The sterilization of media was done by autoclaving at 121 ºC, Petri dishes in case of agar media and in sterilized screw tubes in case of broth media inside laminar hood. The media were incubated at 37 ºC for 24 hours to ensure sterility (Mac Faddin, 2000).

3.2.4. Bacterial identification

3.2.4.1. Morphologic and Microscopic Identification

The phenotypic properties of bacteria were studied in terms of shape, color and consistency and one colony was purified for microscopic diagnosis using light microscopy after being dyed with gram stain, in order to identify the cell forms and clusters.

3.2. 4. 2. Biochemical Test

Biochemical test procedure for identification of bacteria was done according to (Shields *et al*., 2010) and (Pelczar *et al*., 1986).

3.2. 4. 3. Bacterial Identification Using Vitek-2 Compact System

Bacterial suspension was prepared according to the manufacturer's recommendation. A sufficient number of colonies was collected from overnight pure culture transferred and suspending in 3.0 ml of sterile saline in a 12x 75 mm clear (polystyrene) test tube. Turbidity was adjusted to 0.5 McFarland . Using turbidity meter called Densi-chek. The same suspension was used in GN-ID with vitek-2 compact system. Finally GN-ID cassete was loaded to the vitek-2 chamber together with the specimen suspension tubes (Karagoz *et al*., 2015).

3.2.5. Flow Cytometer Procedure

1- Fifty µl of anticoagulant (EDTA) whole blood was added to the bottom of a 12x75 mm polystyrene tube, along with conjugated antibody(s), as directly by manufacturers product insert. Vortex and incubated in the dark at room temperature for the time specified.

2- One hundred µl of reagent A was added to each sample and vortex. The sample was incubated for 10 minutes at room temperature in the dark.

3- One ml of reagent B was added to each sample and vortex. The samples were incubated for 20 minutes in the dark.

4-The samples were centrifuged at 300 x g for 5 minutes. Pour off supernatant and resuspended in1 ml of PBS.

5- The samples were centrifuged at 300 x g for 5 minutes. Pour off supernatant and resuspended in 300 ml of PBs, (this should contain 1 paraformaldehyde for preservation of samples that will not be analyzed the same day).

6- The samples were analyzed on the flow cytometer or stored at 2-8 ºC in the dark until analysis. (Samples can be run up to 24 hours after lysis).

3.2.5.1. Flow cytometer analysis of B cell marker

To analyze the expression of CD19, DR, DQ markers on B cells, the following monoclonal Abs were used: anti-CD19, anti-DR, and anti-DQ. Express CD19 (APC), DR (PE), DQ (FITC) when analyze by flow cytometer.

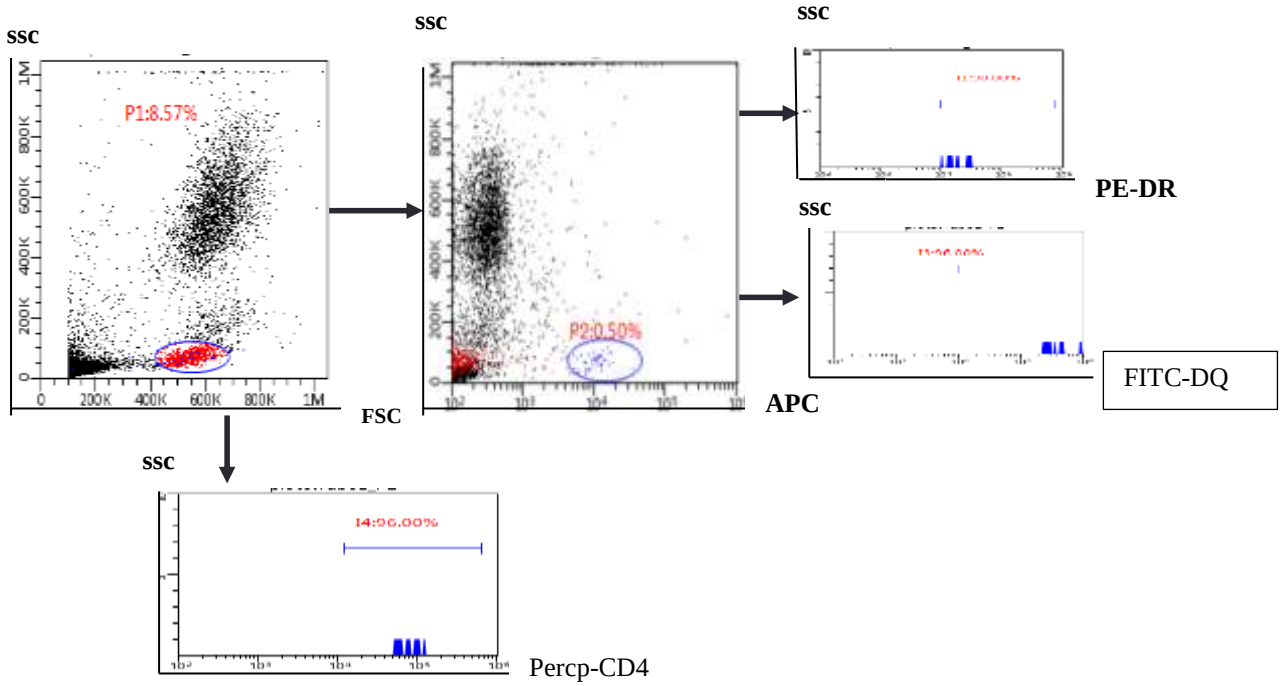


Figure (3-1) Gating strategy for B cell marker

3.2.6. DNA Extraction

Chromosomal DNA was extracted from fresh overnight culture grown in a brain heart broth at 37 ºC, using a GenElute Bacterial Genomic DNA kit (Sigma-USA).

3.2.6.1. DNA Extraction Protocol

A- Cells Harvest

One point five ml of bacterial broth culture was centrifuge at 12,000 xg for 2 minutes; the culture media were completely removed and discarded.

B- Resuspend Cells

The pellet was resuspended in 180 µL of lysis solution T. 20 µL of RNase A was added and incubated for 2 minutes at room temperature.

C-Prepare for cell lysis

Twenty µL of the proteiase K solution were added to the sample, mixed and incubated for 30 minute at 55 ºC.

D-Lyse cells

Two hundred µL of the lysis solution C were added , mixed by vortexing thoroughly for 15 seconds and incubated at 55 ºC for 10 minutes.

E-Column preparation

Five hundred µL of the column preparation solution was added to each pre-assembled GenElute Miniperp binding column in a 2 ml collection tube was seated. Centrifuged at 12,000 x g for 1 minute.

F-Prepare for Binding

Two hundred μL of ethanol (95-100 %) was added to the lysate cells, mixed with inverted for 5-10 seconds. A homogeneous mixture is essential.

G-Load Lysate

The entire content of the tube was transferred into the binding column. Centrifuged at 13,000 x g for 1 minute. The collection tube containing the elute was discarded and the column was placed in a new 2 ml collection tube.

h-First wash

Five hundred μL of wash solution 1was added to the column. Centrifuged at 13,000 x g for 1 minute. The collection tube containing the eluate was discarded and the column was placed in a new 2 ml collection tube.

j- Second Wash

Five hundred μL of wash solution 1 was added to the column. Centrifuged at 12,000 x g for 3minutes to dry column. Finally the collection tube containing the eluate was discarding and the column was placed in a new 2 ml collection tube.

k-Elute DNA

Two hundred μL of the Elution solution was added directly to the center of the column. Centrifuged at 13,000 x g for 1 minute to Elute the DNA.

3.2.7. Protocol of Gel Electrophoresis

3.2.7.1. Agarose Gel Electrophoresis

The well-forming comb was placed in position on casting tray. The open ends of the casting tray were sealed with movable gates. Melted agarose concentration 0.8%, provided with 0.5 mml of red safe dye, and was poured into the casting tray until the agar surrounded the teeth. The gel was hard for about 20 minutes. When the gel was solid, the comb was removed carefully and then end gates lowered.

The buffer chamber was filled with 0.5 X TBE buffer. The buffer was added to cover the gel. The agar was placed in buffer chamber.

The wells were filled with 10 μL samples with 2 ml of loading buffer to be tested and DNA ladder as a size marker in parallel with the sample. The lid was closed and current (70 v) applied for 20 min (for genomic DNA) and 30 min (for PCR product). The power was turned off when the bromophenole blue dye was 5 cm apart the gel was removed (Sambrook *et al*., 1989).

3.2.7.2. Agarose Gel Photo documentation

Agarose gel was visualized in a UV translluminator provided with gel documentation until Agarose gel was placed above the UV translluminator device, than the gel was exposed to UV light 254nm.Then, a picture was taken.

3.2.8. Polymerase Chain Reaction Amplification

Table (3-5): Master Mix composition of Monoplex PCR working solution for *CsgA* gene in each PCR tube

Component	Amount (µL)
Master mix	12.5 µL
DNA	3 µL
Primer	2 µL
Deionized water	7.5 µL
Total volume	25 µL

3.2.9. Thermal Cycling Conditions

Amplification was performed in an automated thermocycler, to detect the presence of *CsgA* gene. Monoplex PCR protocol for csgA gene detection showed in table (3-6)

Table (3-6): Cycling Condition of *CsgA* Gene Amplification

Cycle	Step	Temperature	Time
1cycle	Initial denaturation	94	2 min
30cycle	Denaturation	94	20 sec
	Annealing	62.6	20 sec
	Extension	72	30 sec
1cycle	Final extension	72	5 min
	Hold	4	

3.2.10. Enzyme- Linked Immuno Sorbent Assay (ELISA) protocols for interleukins

For detection of IL-10, IL-23 in serum samples, commercially available human IL-10 and IL-23 mini ELISA development kit was used.

1- The standard working solution was added to the first two columns each concentration is added in duplicate to one well each, side by side (100 µL for each well) and added the samples to other wells (100 µL for each well).

2- The plate was incubated for 90 min at 37 ºC.

3- The liquid was removed out of each well, immediately 100 µL of biotinylated detection Ab working solution was added to each well, covered with the plate sealer.

4- The plate was incubated for1 hour at 37 ºC.

5- Each well was aspirated or decant from the solution, 350 µL of wash buffer was added to each well, soaked for 1-2 minutes and aspirate or decant the solution from each well, and this process was repeated three times in total.

6- One hundred µL of HRP conjugate working solution was added to each well and covered with the plate sealer.

7- The plate was incubated for 3 minutes for 37 ºC.

8- The solution was aspirated or decant from each well. The wash process was repeated for five times.

9- Ninety µL of substrate reagent was added to each well and covered with new plate sealer.

10 - The plate was incubated for 15 minutes at 37 ºC. Protected the plate from light.

11-Fifty μL of stop solution were added to each well.

1 2- The optical density (OD value) determined for each well at once. The microplate reader was set to 450 nm.

3.2.11. Statistical analysis

Statistical analysis was done by using the software statistical package for social science (SPSS; version 22). The results were given as mean ± Standard Error (Mean ± S.E). Statistical analysis for the significance of differences of the quantitative data was done by using independent-sample T test. The probability levels were indicated as follows (one sign $P < 0.05$, two signs $P < 0.01$, three signs $P < 0.001$ and four signs $P < 0.0001$) (Newman *et al*., 1997).

CHAPTER FOURE

4. Results and Discussion

4.1. Demographic distribution of samples groups according to age

Sixty samples were obtained, forty sample patients and twenty samples as control, patients and control in the current study included just females. The patient`s age ranges between 20 to 59 as shown in table (4-1).

Table 4-1: Demographic distribution of samples groups according to the age.

Groups	No.	%	Age categories (years)			
			20 -39		40-59	
			No.	%	No.	%
Control	20	33.33	13	35.14	7	30.43
Patient	40	66.67	24	64.86	16	69.57
Total	60	100	37	100	23	100

Patient samples were divided into two groups according to culture results, groupA patients with bacterial infection and groupB patients without bacterial infection as shown in table (4-2).

Table 4-2: Demographic distribution of samples groups according to age.

Groups	No.	%	Age categories (years)			
			20 -39		40-59	
			No.	%	No.	%
Control group	20	33.33	13	35.14	7	30.43
Patient group A	15	25.00	11	29.72	4	17.40
Patient group B	25	41.67	13	35.14	12	52.17
Total	60	100	37	100	23	100

4.2. Bacterial detection

4.2.1. Identification of bacterial isolates using vitek 2

The Bacterial isolates were identified by vitek 2 automated compact system with GN ⁄ ID card with 64 biochemical tests. The result was identified after 2 to 8 hours as of *Escherichia coli, Klebsiella pneumonia, Raoutltella orintholytica*, *pseudomonas lutellae,* and *Staphylococcus haemolyticus* with probability of 99 %, 99 %, 91 %, 96 % and 87 % respectively (Appendix I).

In current study 25 % of SLE patients were observed to have UTI which was distributed as 53.3 % with *E. coli*, 13.3% with *K. pneumoniae*, 13.3 % with *Proteus spp,* 6.6 % with *P. lutellae* and 6.6 % with *R. orintholytica*.

With regard to aim of this study, it needed to find how bacterial infection in autoimmune disease play role. Infection and SLE are equivalent because they both cause immune-system reactions , but one function protects the Body while the other one allow to do damage to the body (Jung and Suh, 2017).

One of the routes involved is the urinary tract as an infection site in a significant amount of human numbers, particularly females, UTI is also supposed to be involved in systemic autoimmunity as a route that takes the infectious pathogen into the immune system (Doria *et al*., 2008).

Infection is a frequent and severe morbidity and cause of death in patients with SLE. These patients' immune function against bacteria, viruses and fungi has been found to be compromised, and opportunistic or serious infections often occur (Jung *et al*., 2019).

It has been proposed that infectious agents play a pivotal role in individuals with genetic predisposition; these pathogens induce aberrant innate and adaptive immunity, leading to a loss of tolerance towards autoantigens (Pan *et al*., 2019).

4.3. Molecular detection by Polymerase Chain Reaction

4.3.1. Curli gene detection (*CsgA*)

Amplification of the *CsgA* gene of bacterial isolates with primer designated CsgA F, CsgA R showed that the entire DNA samples which extracted from patients group A generated a PCR product of the expected size 178 bp (Figure 4-1).

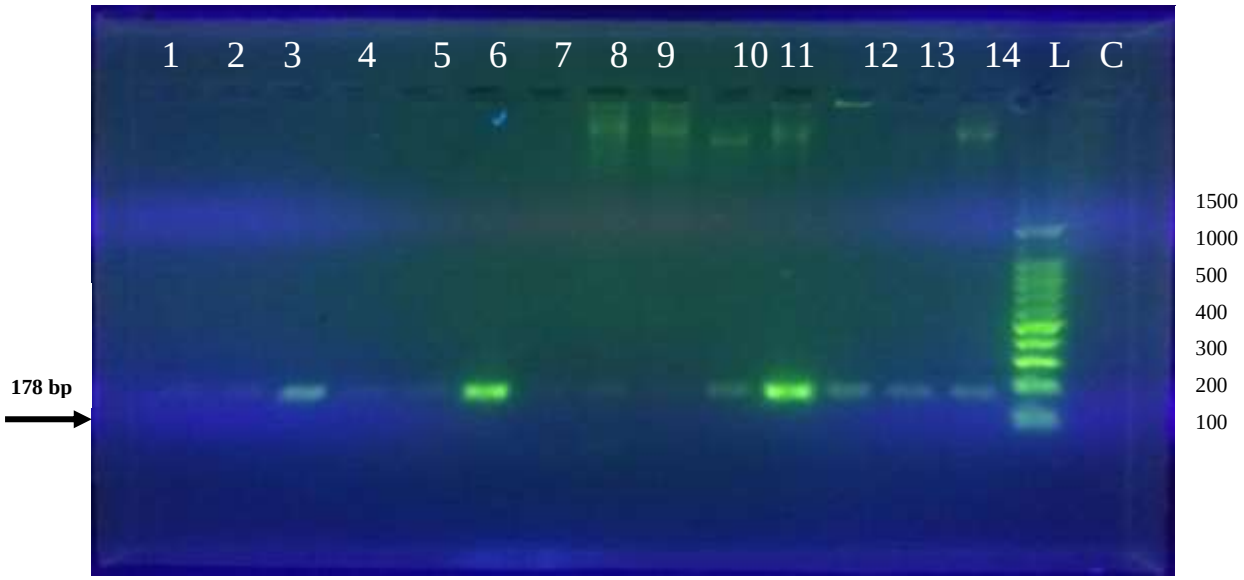


Figure (4-1): Electrophoresis of agarose gel for PCR, a result of *CsgA* gene.. 1.5% agarose, visualized under UV after staining with red safe at 6 volt/cm for 1 hours. Lanes 1 to 14 patients group A. Lane L is DNA ladder (bp), Lane C is negative control.

In the current study the result of PCR showed that all bacterial samples csgA were positive. Curliated pathogens can bypass immune defense and cause inflammation. Interestingly, multiple immune cells such as macrophages, dendritic cells and T cells respond by upregulating various

proinflammatory cytokines including IL-6, IL-23, IL-17A and IL-22 when curliated cells cross the epithelial barrier(Nishimori *et al*., 2012).

Curli also play a part in the development of urinary tract infection by *E. coli*. Curliated uropathogenic E. coli (UPEC) have improved survival relative to non-curliated variants during co-incubation with bladder epithelial cells and this relationship tends to be due to curli interactions with the human antimicrobial peptide LL-37 (Kai-larsen *et al*., 2010). IL-37 usually disturbs membranes that induce lysis but the presence of polymerized curli fibers results in antimicrobial activity inhibition.

The progression of SLE is accelerated by curli-DNA complexes present in enteric bacterial biofilms or curli-expressing bacteria infection. in murine models by producing antibodies to dsDNA and IFN type I response(Gallo *et al*.,2015).

In an experimental UTI model in mice, curli fibers have played a role in bladder colonization at 6 hours after infection (Cegelski *et al*., 2009). Deletion of *csgA* gene in a prototype uropathogenic *E. coli*, in this study, at 6 hours post-infection, resulted in decreased bladder colonizations. Based on these observations, curli fibers in human urinary tract infections (UTIs) have been suggested to be a virulence factor (Norinder *et al*., 2012).

Mice that have been exposed to curli-deficient mutant *E. coli* have not developed autoAbs at all, indicating that exposure to curli amyloid or bacterial infection that can make biofilms with curli / DNA complexes stimulate autoantibodies in susceptible mice (Gallo *et al*., 2015).

Identifying the preserved bacterial factors, DNA and curli that contribute to the response of type IFN and the production of autoantibodies not only provides insight into the pathogenesis of biofilm-associated disease but also sheds light on the interplay between infections and complex human diseases such as SLE (Tursi *et al*., 2017)

4.4. Sero-immunological tests

4.4.1. Blood Human Leukocyte Antigen (DQ) levels Measurement

4.4.1.1. Blood Human Leukocyte Antigen (DQ) Level Measurement According To Groups

Human leukocyte antigen (DQ) levels were detected in blood of patients and controls using flowcytometry technique. The mean level of HLA-DQ was (69.76 ± 3.00) in patients group and (75.62 ± 2.98) in the control group. The level of HLA–DQ decreased in the patients group than control group but without significant difference (Table 4-3, figure 4-2).

Table 4-3: Mean florescent intensity (MFI) of DQ in blood sample of control group, and patient group.

Marker	Group	No.	%	Mean ± S.E.	t-test	D.F	P.
DQ	Control group	20	33.33	75.62 ±2.98	1.23	58	0.22
	Patients group	40	66.67	69.76 ±3.00			

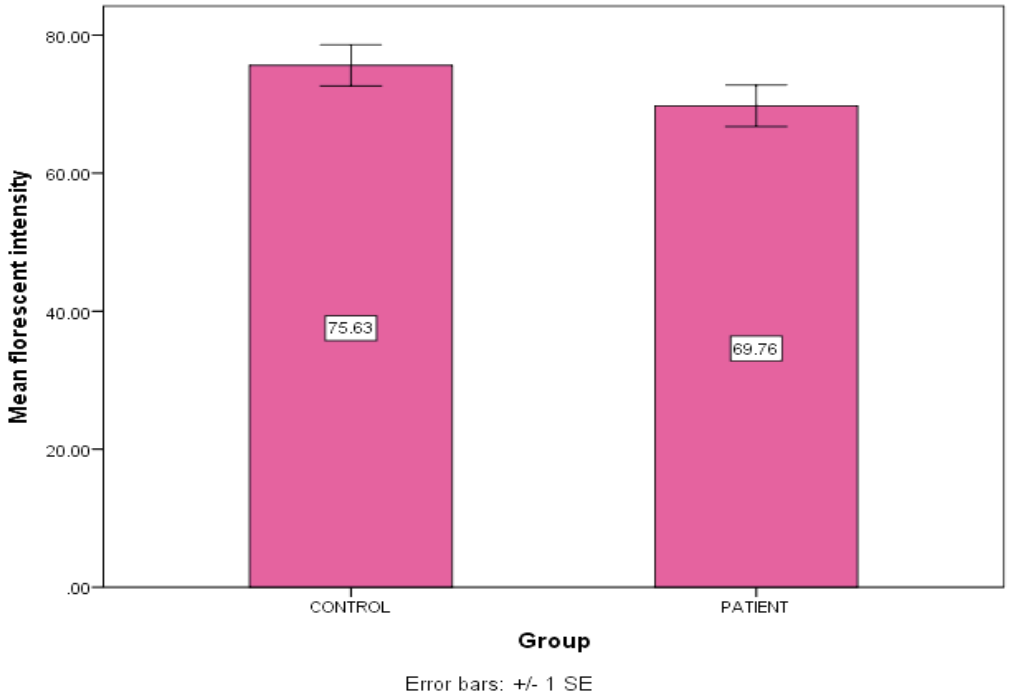


Figure 4-2: Mean florescent intensity of DQ in blood samples of control group, patients group. The significance value was indicated as * between control group and patients group (* P ≤ 0.05, ** P≤ 0.01, *** P ≤ 0.001 and **** P ≤0.0001).

In the current study, the level of HLA-DQ in patients group A, patients group B and control has been investigated. In patients group A mean level was (74.04 ± 4.08) and in patients group B was (67.19 ± 4.11). Thus the level of HLA-DQ in patients group A was higher than those group B but did not reach to the significant level, as shown in table (4-4) and figure (4-3).

Table 4-4: Mean florescent intensity of DQ in blood sample of control group, patients group A and patients group B

Marker	Comparisons Series	Group	No	%	Mean ±S.E.	T Test	D.F	P.
DQ	1	Control group	20	33.33	75.62±2.98	0.32	33	0.75
		Patients group A	15	25.00	74.04±4.08			
	2	Control group	20	33.33	75.62±2.98	1.58	43	0.12
		Patients group B	25	41.67	67.19±4.11			
	3	Patients group A	15	25.00	74.04±4.08	1.10	38	0.27
		Patients group B	25	41.67	67.19±4.11			

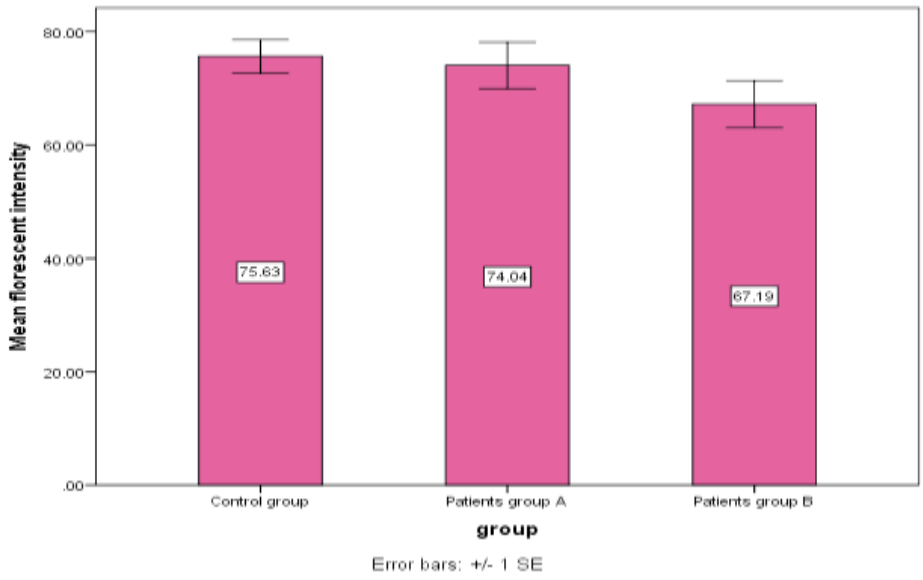


Figure 4-3: Mean florescent intensity of DQ in blood samples of control group, patients Group A and patients Group B. The significance value was indicated as * between control group and patients group A, ● between control group and patients group B, and ▲ between patients group A and patients group B, The level of probability was indicated as (* ,●, ▲ P ≤ 0.05, **, ●●,▲▲ P≤ 0.01, *, ●●●,▲▲▲ P ≤ 0.001 and ****, ●●●●, ▲▲▲▲ P ≤0.0001).**

4.4.1.2. Blood Human Leukocyte Antigen (DQ) Level According To Age Categories

Among comparison between patients group and control group according to age, the level of HLA-DQ has been investigated. The mean level of HLA-DQ among the age categories did not reach to the significant level (Table 4-5 and Figure 4-4).

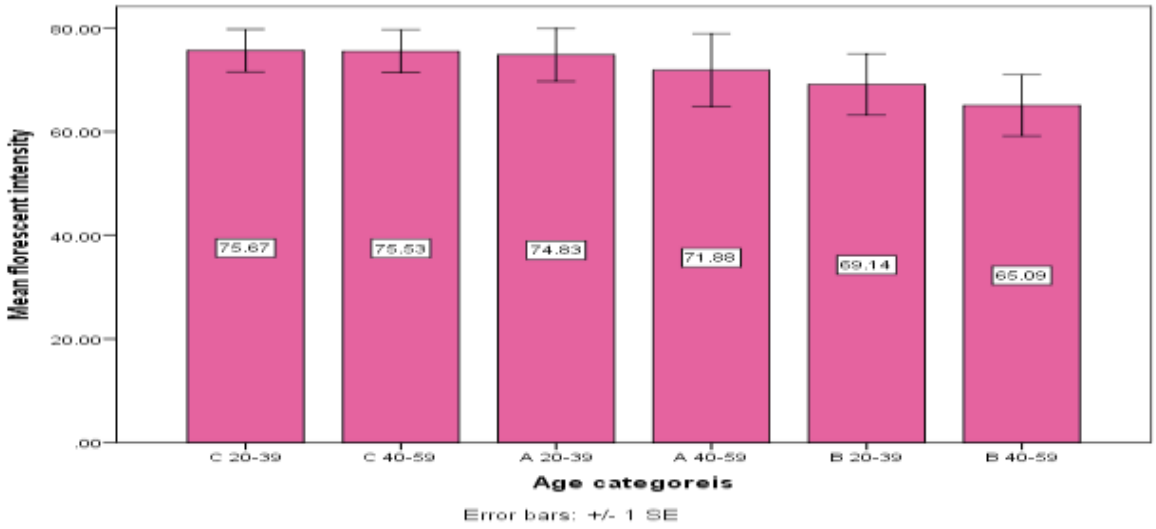


Figure (4-4): Mean florescent intensity of DQ in blood sample of age categories of control group, patients group A and patients group B. The significance value was indicated as * between 20-39 category of control group and 20-39 category of patients group A , ◦ between 20-39 category of control group and 20-39 category of patients group B. (The level of probability was indicated as*,◦ P ≤ 0.05, **,◦◦ P ≤ 0.01, *,◦◦◦ P ≤ 0.001 and ****,◦◦◦◦P ≤ 0.0001).**

Table 4-5: Mean florescent intensity of DQ in blood samples of age categories of control group, patients group A and patients group B

Marker	Comparisons Series	Group	No.	%	Mean ± S.E.	t-test	D.F.	P.
DQ	1	Control group 20-39	13	21.67	75.67±4.12	0.02	18	0.98
		Control group 40-59	7	11.66	75.53 ±4.16			
	2	Patients group A 20-39	11	18.33	74.83±5.11	0.30	13	0.76
		Patients group A 40-59	4	6.67	71.87±7.05			
	3	Patients group B 20-39	13	21.67	69.13±5.90	0.48	23	0.63
		Patients group B 40-59	12	20.00	65.08±5.93			
	4	Control group 20-39	13	21.67	75.67±4.12	0.13	22	0.89
		Patients group A 20-39	11	18.33	74.83±5.11			
	5	Control group 20-39	13	21.67	75.67±4.12	0.90	24	0.37
		Patients group B 20-39	13	21.67	69.13±5.90			
	6	Patients group A 20-39	11	18.33	74.83±5.11	0.71	22	0.48
		Patients group B 20-39	13	21.67	69.13±5.90			
	7	Control group 40-59	7	11.66	75.53±4.16	0.48	9	0.64
		Patients group A 40-59	4	6.67	71.87±7.05			
	8	Control group 40-59	7	11.66	75.53±4.16	1.23	17	0.23
		Patients group B 40-59	12	20.00	65.08±5.93			
	9	Patients group A 40-59	4	6.67	71.87±7.05	0.60	14	0.55
		Patients group B 40-59	12	20.00	65.08±5.93			

4.4.2. Blood Human Leukocyte Antigen (DR 4) levels measurement

4.4.2.1. Blood Human Leukocyte Antigen (DR4) Level measurement According To groups

Human leukocyte antigen (DR4) levels were detected in blood of patients and control using flowcytometry technique. The mean level of HLA-D R4 was (76.30 ± 3.53) in patients group and (89.35 ± 1.93) in the control group. The level of HLA-DR4significant decreased in the patients group compared to control group as shown in Table (4-6) and Figure (4-5).

Table 4-6: Mean florescent intensity of DR4 in blood sample of control group, and patients group.

Marker	Group	No.	%	Mean ± S.E.	t-test	D.F	P.
DR4	Control group	20	33.33	89.35 ± 1.93	2.50	58	0.015
	Patients group	40	66.67	76.30 ± 3.53			

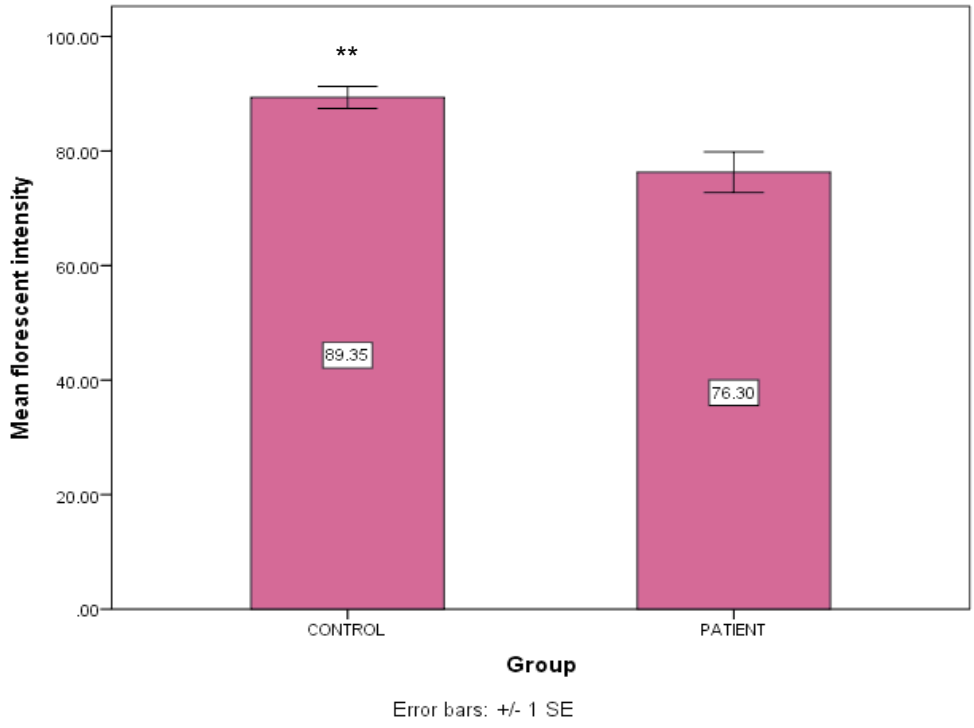


Figure 4-5: Mean florescent intensity of DR4 in blood sample of control group and patients group. The significance value was indicated as * between control group and patients group, (the level of probability was indicated as * P ≤ 0.05, ** P ≤ 0.01. *** P ≤ 0.001 and ****P ≤ 0.001).

In this study, the level of HLA-DR in patients group A, patients group B and control have been investigated. In patients group A mean level was (68.68 ± 7.46) and in patients group B was (80.87 ± 3.27), thus the level of HLA-DR in patients group A significantly decreased compared to control group, furthermore there were differences in the level of HLA-DR in patients group A than patients group B but without significant difference (Table 4-7, Figure 4-6).

Table 4-7: Mean florescent intensity of DR4 in blood sample of control group, patients group A and patients group B

Marker	Comparisons Series	Group	No.	%	Mean ± S.E.	t-test	D.F	P.
DR4	1	Control group	20	33.33	89.35±1.93	3.03	33	0.005
		Patients group A	15	25.00	68.68±7.46			
	2	Control group	20	33.33	89.35±1.93	2.09	43	0.042
		Patients group B	25	41.67	80.87±3.27			
	3	Patients group A	15	25.00	68.68±7.46	1.70	38	0.096
		Patients group B	25	41.67	80.87±3.27			

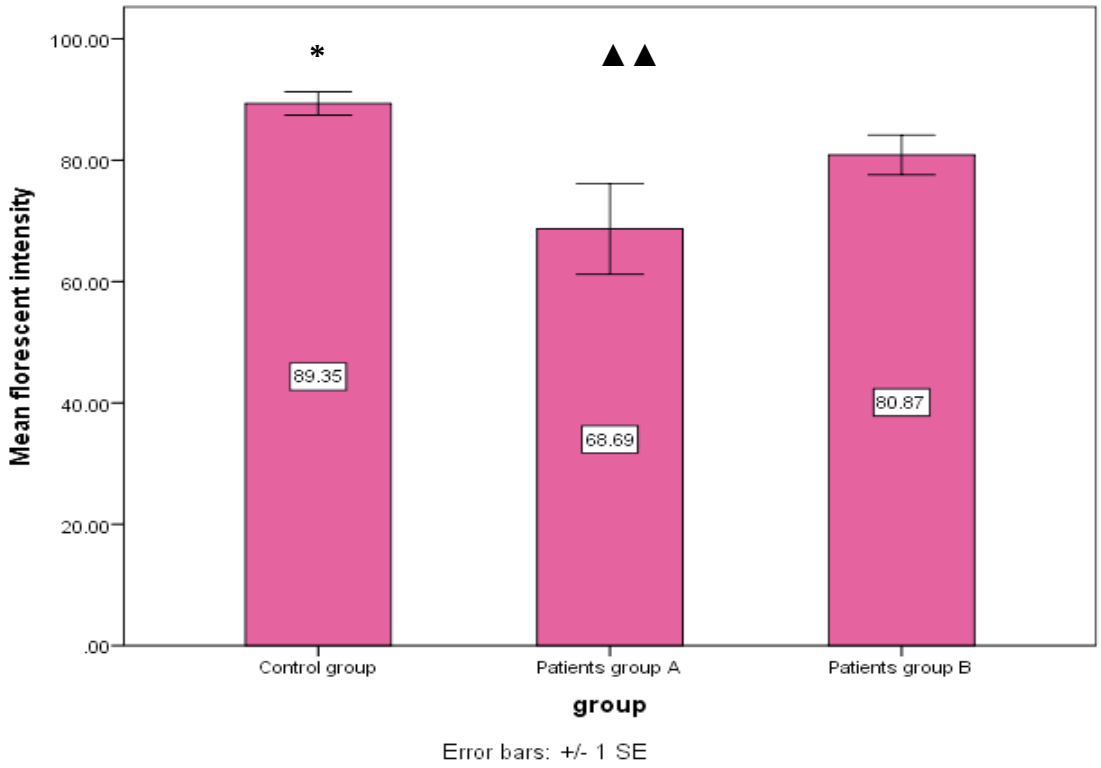


Figure 4-6: Mean florescent intensity of DR in blood sample of control group, patients Group A and patients Group B. The significance value was indicated as * between control group and patients group A, ● between control group and patients group B and ▲ between patients group A and patients group B, The level of the probability was defined as (* ,●, ▲ P ≤ 0.05, **, ●●,▲▲ P≤ 0.01, *, ●●●,▲▲▲ P ≤ 0.001 and ****, ●●●●, ▲▲ P ≤0.0001).**

4.4.2.2. Blood Human Leukocyte Antigen (DR 4) Level According To Age Categories

By comparing between patients groups and control group according to age, the level of HLA-DR has been investigated. The mean level of HLA-DR among the age categories showed significantly increased in control group than patients group A and patients group B with age range (20-39), as shown in Figure (4-7) and Table (4-8).

Table 4-8: Mean florescent intensity of DR4 in blood sample of age categories of control group, patients group A and patients group B

Marker	Comparisons Series	Group	No.	%	Mean ± S.E.	t-test	D.F.	P.
DR4	1	Control group 20-39	13	21.67	90.80 ± 2.21	1.02	18	0.32
		Control group 40-59	7	11.66	86.65 ± 3.69			
	2	Patients group A 20-39	11	18.33	67.53 ±9.80	0.24	13	0.809
		Patients group A 40-59	4	6.67	71.85 ± 9.47			
	3	Patients group B 20-39	13	21.67	80.22 ± 4.85	0.20	23	0.842
		Patients group B 40-59	12	20.00	81.57 ± 4.55			
	4	Control group 20-39	13	21.67	90.80 ± 2.21	2.50	22	0.020
		Patients group A 20-39	11	18.33	67.53 ±9.80			
	5	Control group 20-39	13	21.67	90.80 ± 2.21	1.98	24	0.059
		Patients group B 20-39	13	21.67	80.22 ± 4.85			
	6	Patients group A 20-39	11	18.33	67.53 ±9.80	1.21	22	0.237
		Patients group B 20-39	13	20.00	80.22 ± 4.85			
	7	Control group 40-59	7	11.66	86.65 ± 3.69	1.74	9	0.115
		Patients group A 40-59	4	6.67	71.85 ± 9.47			
	8	Control group 40-59	7	11.66	86.65 ± 3.69	0.76	17	0.455
		Patients group B 40-59	12	20.00	81.57 ± 4.55			
	9	Patients group A 40-59	4	6.67	71.85 ± 9.47	1.02	14	0.325

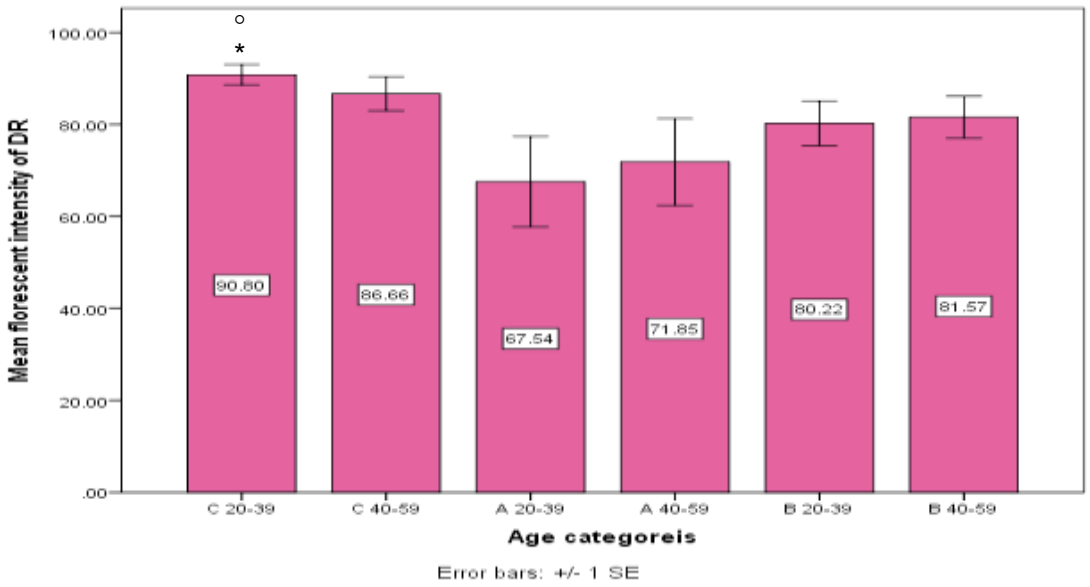


Figure 4-7:Mean florescent intensity of DR in blood sample of age categories of control group, patients group A and patients group B. The significance value was indicated as * between 20-39 category of control group and 20-39 category of patients group A , ◦ between 20-39 category of control group and 20-39 category of patients group B. (The level of probability was indicated as*,◦ P ≤ 0.05, **,◦◦ P ≤ 0.01, *,◦◦◦ P ≤ 0.001 and ****,◦◦◦◦P ≤ 0.0001).**

In the current study the level of HLA-DR on B cells in SLE patients decreased in comparison with control and the level of these molecules decreased in patients with infection than patients without infection. These molecules should be increased in response to bacterial infection in persons who have immune system without any defective but in the current study patients severe from type of autoimmune disease that cause defect to immune system.

Variations in the role of APCs associated with SLE includes decreased development of IL-1, decreased expression of the HLA-DR surface membrane, decreased ability to mediate autologous mixed lymphocyte reaction (MLR), and decreased *in vitro* receptor activity for the Fc portion of IgG (Tsokos *et al*., 1996)

Related with the SLE disease immune-regulatory dysfunctions, two different mechanisms could lead to a reduction of DR antigens. Firstly, autoantibodies blocking the surface antigens cause an apparent reduction in DR molecules, and secondly, System for DR-producing malfunctions, That includes the gene itself,The decreased amount of DR molecules will account for , The first is based on the finding that anti-leukocyte antibodies are developed by SLE patients (Sano *et al*., 1985). A decrease in HLA-DR expression was not only observed in critically ill patients, But even in patients suffering from autoimmune disorders including systemic lupus erythematosus (Tillinger *et al*., 2013).

HLA-DR is decreased or defective, this event upregulates the surface expression of negative co-stimulatory molecules programmed death 1 (PD-1), cytotoxic T-lymphocyte antigen 4 (CTLA-4), B- and T-lymphocyte attenuator (BTLA) and their corresponding ligands, such as PD-1 (PD-L1) ligands. These acts, including differentiation cluster (CD) 8 and anergic CD4-positive T cells, can compromise innate and adaptive immune systems and induce T cell apoptosis (Zmijewski *et al*., 2020).

In contrast to HLA-DR, the level of HLA-DQ on B cell increased in patients with infection than patients without infection but did not reach significant level. The result of HLA-DR and DQ expression on B cell in SLE patients with infection that showed increase in HLA-DQ in these patients response to infection with gram negative bacteria that express *csgA* gene but not change in the level of HLA-DR antigen in these patients may related to the effect of SLE disease on HLA-DR cause decrease in this molecule even in presence of infection but HLA-DQ unaffected by this disease.

HLA-DQ is also important in the response to bacterial antigens. Particular DQ genotypes have been shown to have an impaired response to several types of bacterial infections (Taylor *et al*., 2000).

4.4.3. Investigation of CD19+B cells

4.4.3.1. CD19+B cells levels measurement According To groups

CD19+B cells levels were investigated in blood of patients and control using flowcytometry technique. The mean level of CD19+B cells was (3.13 ± 0.15) in patients group and (1.38 ± 0.22) in the control group. The level of CD19+B cells in the patients group significantly increased compared to control group (Table 4-9 and Figure 4-8).

Table 4-9: Percentage of CD19+B cells in blood sample of control and patients group.

Marker	Group	No.	%	Mean ± S.E.	t-test	D.F	P.
CD19	Control group	20	33.33	1.38±0.22	6.4	58	0.0001
	Patients group	40	66.67	3.13±0.15			

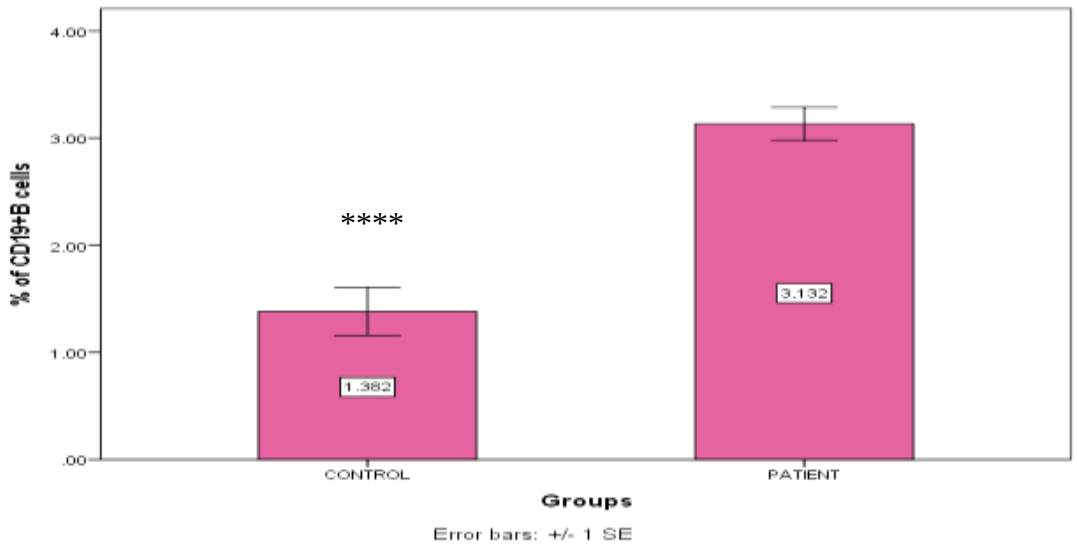


Figure (4-8) Percentage of CD19+B cells in blood sample of control group, and patients group. The significance value was indicated as * between control group and patients group. The level of probability was indicated as (*P ≤ 0.05, **P≤ 0.01, *** P ≤ 0.001 and **** P ≤0.0001).

In this study, the level of CD19+B cells in patients group A, patients group B and control have been investigated. In patients group A mean level was (3.85 ± 0.28), in control group was (1.38 ± 0.22) and in patients group B was (2.7 ± 0.11), thus the level of CD19+B cells in patients group A was significantly increased compared to control group, furthermore there were significant differences in the level of CD19+B cells in patients group A than patients group B (Table 4-10 and Figure 4-9).

Table 4-10: Percentage of CD19+B cells in blood sample of control group, patients group A and patients group.

Marker	Comparisons Series	Group	No.	%	Mean ± S.E.	t-test	D.F.	P.
CD19	1	Control group	20	33.33	1.38±0.22	6.8	33	0.0001
		Patients group A	15	25.00	3.85±0.28			
	2	Patients group A	15	25.00	3.85±0.28	4.3	38	0.0001
		Patients group B	25	41.67	**2.7**±0.11			
	3	Control group	20	33.33	1.38±0.22	5.5	43	0.0001
		Patients group B	25	41.67	**2.7**±0.11			

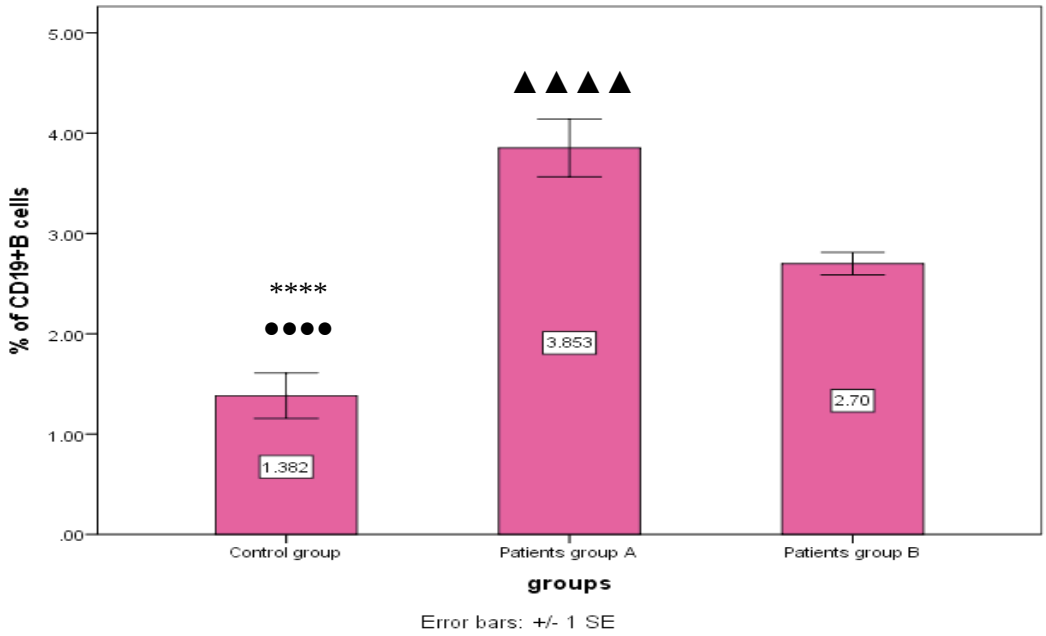


Figure (4-9): Percentage of CD19+B cells in blood sample of control group, patients Group A and patients Group B. The significance value was indicated as * between control group and patients group A, ● between control group and patients group B and ▲ between patients group A and patients group B. The level of the probability was defined as (*, ●, ▲ P ≤ 0.05, **, ●●, ▲▲ P≤ 0.01, *, ●●●,▲▲▲ P ≤ 0.001 and ****, ●●●●, ▲▲▲▲ P ≤0.0001).**

4.4.3.2. CD19+B cells level measurement according to age categories

By comparing between patients group and control group according to age the level of CD19+B cells has been investigated. The mean level of CD19+B cells among the age categories showed significant increase in the level of CD19+B cells in patients group A with age range (20-39) and patients group A with age (40-59) compared to control and patients group B (Figure 4-10 and Table 4-11).

Table 4-11: Percentage of CD19+B cells in blood sample of age categories of control group, patients group A and patients group B

Marker	Comparisons Series	Group	No.	%	Mean ± S.E.	t-test	D.F.	P.
CD19	1	Control group 20-39	13	21.67	1.31±0.28	0.38	18	0.7
		Control group 40-59	7	11.66	1.50±0.39			
	2	Patients group A 20-39	11	18.33	3.51±0.15	2.16	13	0.04
		Patients group A 40-59	4	6.67	4.77±0.92			
	3	Patients group B 20-39	13	21.67	2.72±0.16	0.25	23	0.7
		Patients group B 40-59	12	20.00	2.66±0.15			
	4	Control group 20-39	13	21.67	1.31±0.28	6.42	22	0.0001
		Patients group A 20-39	11	18.33	3.51±0.15			
	5	Control group 20-39	13	21.67	1.31±0.28	4.27	24	0.0001
		Patients group B 20-39	13	21.67	2.72±0.16			
	6	Patients group A 20-39	11	18.33	3.51±0.15	3.46	22	0.02
		Patients group B 20-39	13	21.67	2.72±0.16			
	7	Control group 40-59	7	11.66	1.50±0.39	3.82	9	0.04
		Patients group A 40-59	4	6.67	4.77±0.92			
	8	Control group 40-59	7	11.66	1.50±0.39	3.22	17	0.05
		Patients group B 40-59	12	20.00	2.66±0.15			
	9	Patients group A 40-59	4	6.67	4.77±0.92	3.69	14	0.02
		Patients group B 40-59	12	20.00	2.66±0.15			

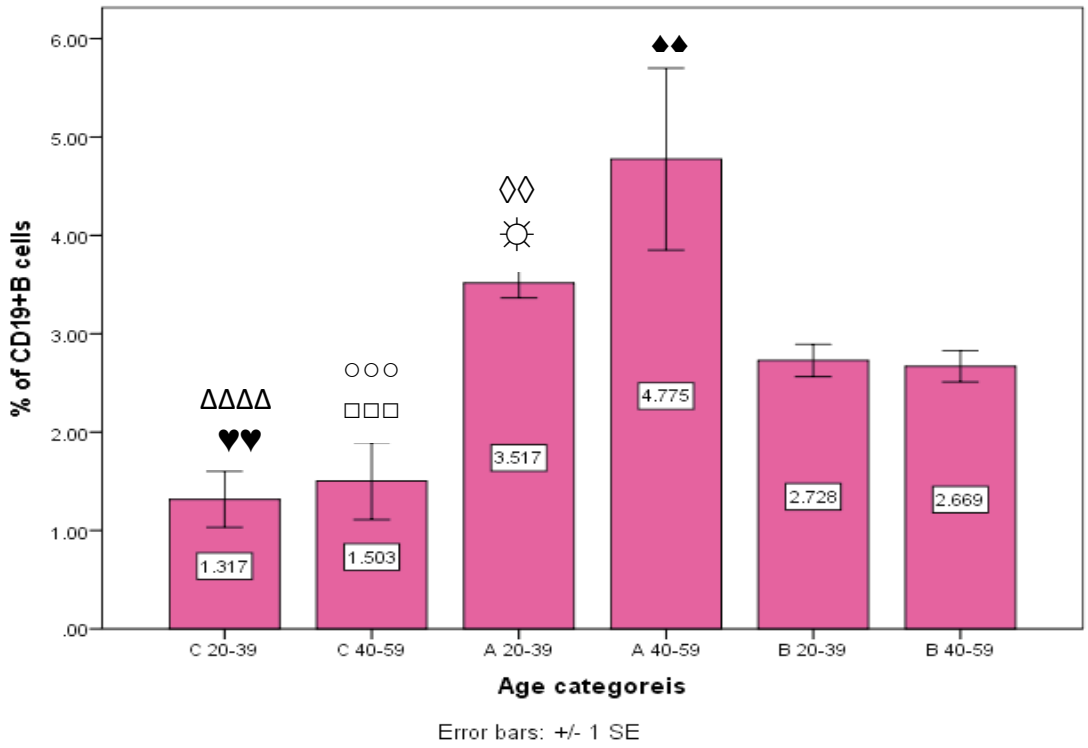


Figure (4-10): Percentage of CD 19+B in age categories of control group, patients group A and patients group B. The significance value was indicated as Δ between 20-39 category of control group and 20-39 category of patients group A, ♥ between 20-39 category of control group and 20-39 category of patients group B, and ◊ between 20-39 category of patients group A and 20-39 category of patients group B, ○ between 40-59 category of control group and 40-59 category of patients group A, □ between 40-59 category of control group and 40-59 category of patients group B, and ♦ between 40-59 category of patients group A and 40-59 category of patients group B,☼ between 20-39 category and 40-59 category of patients group A(the level of probability was indicated as one sign P ≤ 0.05, two signs P ≤ 0.01, three signs P ≤ 0.001 and four signs P ≤ 0.0001).

In current study the results showed increase in the level of CD19+B cells in patients with infection than in those without infection, the results of current study agreed with study published by (Lu *et al*., 2019).

Furthermore, deregulation of the adaptive immune system leads to inadequate response of SLE patients to infections. B and T cells have increased activation with decreased numbers in SLE, SLE CD4+T cells

with decreased Th1 cells resulting in fewer defenses against viruses and intracellular bacteria having a bias for Th2 and Th17 requirements. Patients have also reduced the generation and suppression of regulatory T-cells (Tregs) which promote inflammation (Caza *et al*., 2013).

The basic immune response between men and women was known to differ, with women producing a more vigorous immune response and increased production of antibodies, however autoimmune diseases that develop in men are often more severe. Sex hormones, such as estrogen, testosterone and progesterone, may mediate most of the sex-based differences in the immune response estrogens and androgens have recently been found to directly influence whether an immune response of the Th1 or

Th2 type develops by interacting with hormone receptors on immune cells (Fairweather and Rose, 2004).

4.4.4. CD4+T cells levels measurement

4.4.4. 1. CD4+Tcells levels measurement according to groups

CD4+Tcells levels were detected in blood of patients and control using flowcytometry technique. The mean level of CD4 was (88.44 ± 2.25) in patients group and (92.89 ±1.01) in the control group. The level of CD4 +T cells in the patients group significantly increase compared to control (Table 4-12 and Figure 4-11).

Table 4-12: Percentage of CD4+T cells in blood sample of control and patients group.

Marker	Group	No.	%	Mean ± S.E.	t-test	D.F	P.
CD4	Control group	20	33.33	6.48±0.24	3.7	58	0.0001
	Patients group	40	66.67	8.93±0.44			

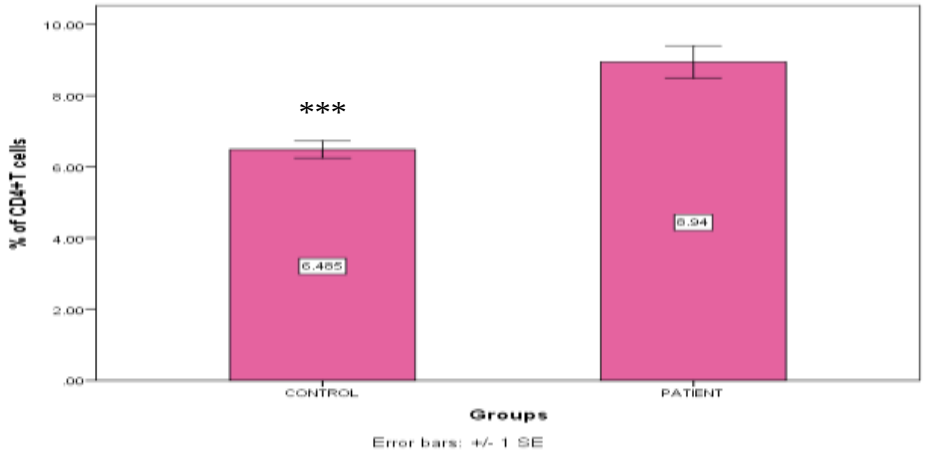


Figure (4-11): Percentage of CD4+T cells in blood sample of control group, and patients group. The significance value was indicated as * between control group and patients group. The level of probability was indicated as (*P ≤ 0.05, **P≤ 0.01, *** P ≤ 0.001 and **** P ≤0.0001).

In this study, the level of CD4+T cells in patients group A, patients group B and control have been investigated. In patients group A mean level was (82.74 ± 5.45) and in patients group B was (91.86 ± 1.22). Thus the level of CD4+T cells in patients group A significantly decrease compared to patients group B and also showed significant decrease in patients group A than control group (Figure 4-12 and Table 4-.13).

Table 4-13: Percentage of CD4+T cells in blood sample of control, patients group A and patients group B

Marker	Comparisons Series	Group	No.	%	Mean ± S.E.	t-test	D.F.	P.
CD4	1	Control group	20	33.33	6.48±0.24	4.7	33	0.0001
		Patients group A	15	25.00	10.51±0.92			
	2	Patients group A	15	25.00	10.51±0.92	2.9	38	0.05
		Patients group B	25	41.67	7.99±0.35			
	3	Control group	20	33.33	6.48±0.24	3.3	43	0.02
		Patients group B	25	41.67	7.99±0.35			

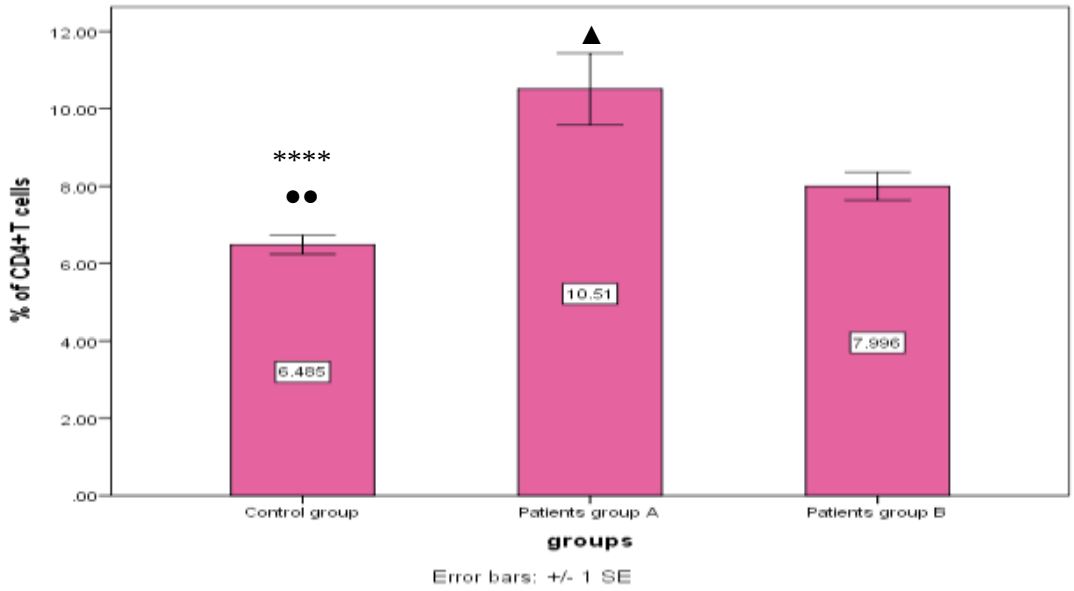


Figure (4-12): Percentage of CD4+T cells in blood sample of control group, patients group A and patients group B. The significance value was indicated as * between control group and patients group A, ● between control group and patients group B and ▲ between patients group A and patients group B, The level of probability was indicated as (*,●, ▲ P ≤ 0.05, **, ●●, ▲▲ P≤ 0.01, *, ●●●, ▲▲▲ P ≤ 0.001 and ****, ●●●●, ▲▲▲▲ P ≤ 0.0001).**

4.4.4.2. CD4 +T cells Level According To Age Categories

By comparing between patients group and control group according to age the level of CD4+T cells has been investigated. The mean level of CD4+Tcells among the age categories showed significant increase in the level of CD4+T cells in patients group A with age range (20-39) and patients group A with age (40-59) compared to control and patients group B (Figure 4-13 and Table 4-14).

Table 4-14: Percentage of CD4+T cells in blood sample of age categories of control group, patients group A and patients group B

Marker	Comparisons Series	Group	No.	%	Mean ± S.E.	t-test	D.F.	P.
CD4	1	Control group 20-39	13	21.67	6.39±0.31	0.49	18	0.6
		Control group 40-59	7	11.66	6.65±0.42			
	2	Patients group A 20-39	11	18.33	9.28±0.43	2.64	13	0.02
		Patients group A 40-59	4	6.67	13.89±2.82			
	3	Patients group B 20-39	13	21.67	8.25±0.62	0.75	23	0.45
		Patients group B 40-59	12	20.00	7.71±0.33			
	4	Control group 20-39	13	21.67	6.39±0.31	5.52	22	0.0001
		Patients group A 20-39	11	18.33	9.28±0.43			
	5	Control group 20-39	13	21.67	6.39±0.31	2.68	24	0.01
		Patients group B 20-39	13	21.67	8.25±0.62			
	6	Patients group A 20-39	11	18.33	9.28±0.43	1.30	22	0.20
		Patients group B 20-39	13	21.67	8.25±0.62			
	7	Control group 40-59	7	11.66	6.65±0.42	3.41	9	0.008
		Patients group A 40-59	4	6.67	13.89±2.82			
	8	Control group 40-59	7	11.66	6.65±0.42	1.94	17	0.06
		Patients group B 40-59	12	20.00	7.71±0.33			
	9	Patients group A 40-59	4	6.67	13.89±2.82	3.81	14	0.02
		Patients group B 40-59	12	20.00	7.71±0.33			

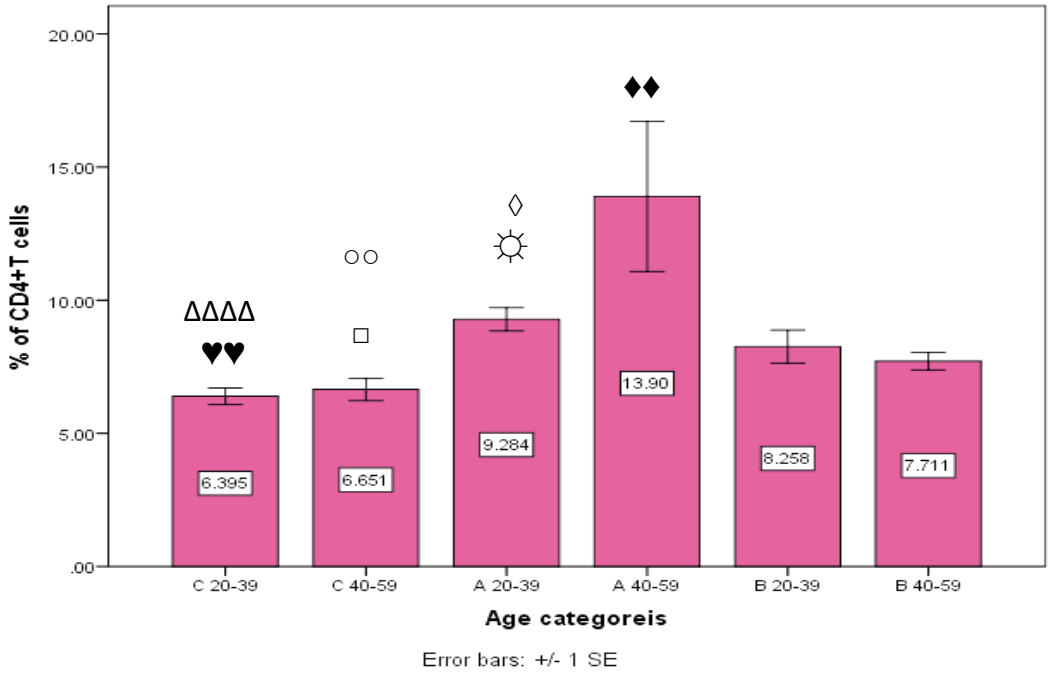


Figure (4-13) Percentage of CD4+T in age categories of control group, patients group A and patients group B. The significance value was indicated as Δ between 20-39 category of control group and 20-39 category of patients group A, ♥ between 20-39 category of control group and 20-39 category of patients group B, and ◊ between 20-39 category of patients group A and 20-39 category of patients group B, ○ between 40-59 category of control group and 40-59 category of patients group A, □ between 40-59 category of control group and 40-59 category of patients group B, and ♦ between 40-59 category of patients group A and 40-59 category of patients group B,■ between 20-39 category and 40-59 category of control group,☼ between 20-39 category and 40-59 category of patients group A, ♯ between 20-39 category and 40-59 category of patients group B. (The level of probability was indicated as one sign P ≤ 0.05, two signs P ≤ 0.01, three signs P ≤ 0.001 and four signs P ≤ 0.0001).

In the control of autoimmunity, immune homeostasis and immune response to pathogens and tumor antigens,CD4+ T cells have been reported to play a key role (Jiang and Dong, 2013).CD4+T cells mediate macrophage activation and thus play a critical role in viral and bacterial control (Swain *et al.*, 2005).

The patients with SLE take immunosuppressive drugs like prednisolone, cyclophosphamide and others. (Wu *et al*., 2018) published report on patients undergo to treat with immunosuppressive drugs divided into patients with and without infection to determine the level of lymphocyte showed the level of CD4 lower in patients with infection than patients without infection. (Lu *et al*., 2019) published another study similar to first study but patients untreated with immunosuppressive drugs the results similar in both studies, the result of last study improve the alter in lymphocyte level related to the nature of autoimmune disease and infection because no immunosuppressive used.

Wu *et al*. reported that the CD4+T cell number and the CD4/CD8 ratio as well as immunoglobulin G level were lower in SLE patients with infection than in those without infection. This study in contrast to current study that showed increased in the CD4+Tcells number in patients with infection than patients without infection, the cause may be due to presence of csgA gene in bacterial samples and this gene may be wasn't present in bacterial samples of Wu *et al*. study. We can conclude that csgA gene cause effect on the adaptive immune system through activation B and T cells that lead to significant increase in the number of CD4+Tcells and CD19+Bcells.

4.4.5. Serum Cytokine Levels Measurement

4.4.5.1. Serum Interleukin-23 (IL-23) Levels Measurement According To Groups

To investigate the role of IL-23 in patients and control the serum levels of this cytokine have been measured. The mean level of IL-23 was (68.19 ± 4.05) in patients group and (46.74 ± 3.83) in the control group. The mean level of IL-23 in patients group was significantly higher than control group (Table 4-15 and Figure 4-14).

Table 4-15: Concentration of IL-23 in blood sample of control group and patient group .

Marker	Group	No.	%	Mean ± S.E.	t-test	D.F	P.
IL-23	Control group	20	33.33	46.74±3.83	3.37	58	0.001
	Patients group	40	66.67	68.19±4.05			

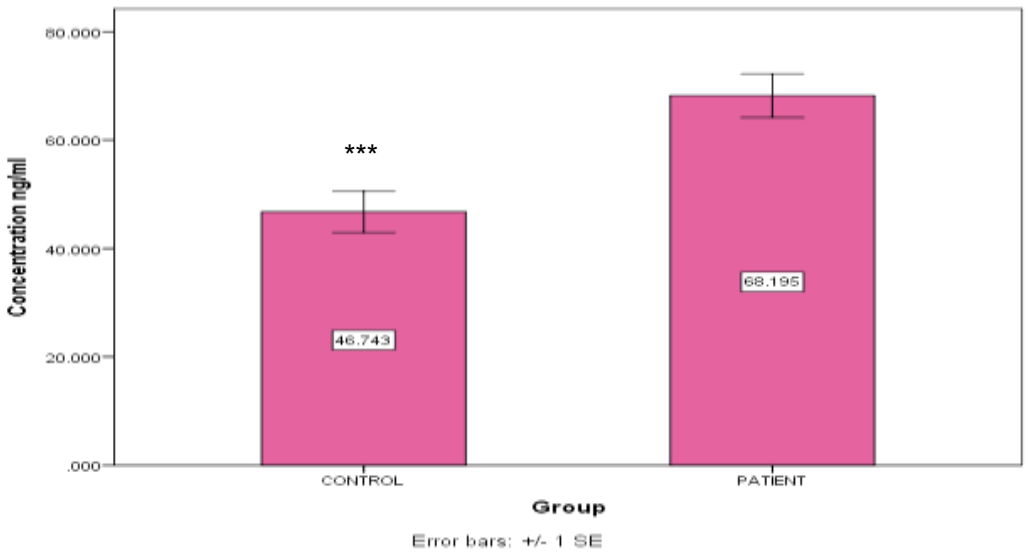
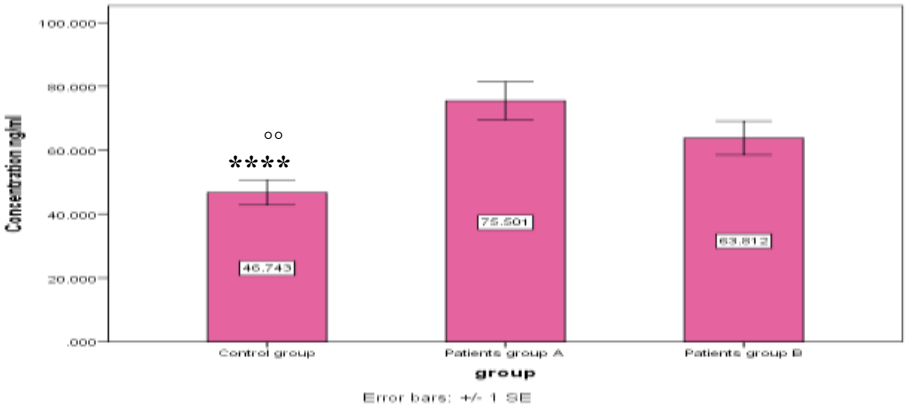


Figure (4-14): Concentration of IL-23 in blood sample of control group and patients group. The significance value was indicated as* between control group and patients group (the level of probability was indicated as * P < 0.05, P < 0.01,*** P < 0.001 and **** P < 0.0001).**

In this study, the level of IL-23in patient group A, patient group B and control have been investigated. In patient group A means level was (75.50±6.03) and in patients group B was (63.81±5.27). Thus the serum levels of IL-23in patients group A significant increase in compared with control group, furthermore the level increase in patients group A than Patients group B but without significant difference (Table 4-16 and Figure4-15).

Table 4-16: Concentration of IL-23 in blood sample of control group, patients group A and patients group B

Marker	Comparisons Series	Group	No.	%	Mean ± S.E.	t-test	D.F.	P.
IL-23	1	Control group	20	33.33	46.74±3.83	4.20	33	0.0001
		Patients group A	15	25.00	75.50±6.03			
	2	Control group	20	33.33	46.74±3.83	2.49	43	0.01
		Patients group B	25	41.67	63.81±5.27			
	3	Patients group A	15	25.00	75.50±6.03	1.41	38	0.16
		Patients group B	25	41.67	63.81±5.27			



Figure(4-15): Concentration of IL-23 in blood sample of control group, patients GroupA and patients GroupB. The significance value was indicated as * between control Group and patients Group A, ◦ between control Group and patients Group B, (the level of probability was indicated as *, ◦ P ≤ 0.05, **,◦◦ P ≤ 0.01, * ,◦◦◦ P ≤ 0.001 and **** ,◦◦◦◦ P ≤ 0.0001).**

4.4.5.2. Serum interleukin-23(IL-23) levels measurement according to age categories

By comparing between patients group and control group according to age, the level of IL-23has been investigated. The mean level of IL-23 among the age categories was significantly increase in patients group A age range (20-39) compared to control group with same age range and in patients group A age range (40-59) the level significantly higher when compared with controls group (40-59) (Table 4-17 and Figure 4-16).

Table 4-17: Concentration of IL-23 in blood sample of age categories of control group, patients group A and patients group B

Marker	Comparisons Series	Group	No.	%	Mean ± S.E.	t-test	D.F.	P.
Il23	1	Control group 20-39	13	21.67	45.48 ± 5.07	0.43	18	0.66
		Control group 40-59	7	11.66	49.07 ± 5.99			
	2	Patients group A 20-39	11	18.33	66.96 ± 4.53	2.89	13	0.01
		Patients group A 40-59	4	6.67	98.96 ± 14.07			
	3	Patients group B 20-39	13	21.67	66.45 ± 4.82	0.51	23	0.61
		Patients group B 40-59	12	20.00	60.94 ± 9.88			
	4	Control group 20-39	13	21.67	45.48 ± 5.07	3.10	22	0.05
		Patients group A 20-39	11	18.33	66.96 ± 4.53			
	5	Control group 20-39	13	21.67	45.48 ± 5.07	2.99	24	0.06
		Patients group B 20-39	13	21.67	66.45 ± 4.82			
	6	Patients group A 20-39	11	18.33	66.96 ± 4.53	0.07	22	0.94
		Patients group B 20-39	13	21.67	66.45 ± 4.82			
	7	Control group 40-59	7	11.66	49.07 ± 5.99	3.82	9	0.04
		Patients group A 40-59	4	6.67	98.96 ± 14.07			
	8	Control group 40-59	7	11.66	49.07 ± 5.99	0.85	17	0.40
		Patients group B 40-59	12	20.00	60.94 ± 9.88			
	9	Patients group A 40-59	4	6.67	98.96 ± 14.07	3.82	14	0.04
		Patients group B 40-59	12	20.00	60.94 ± 9.88			

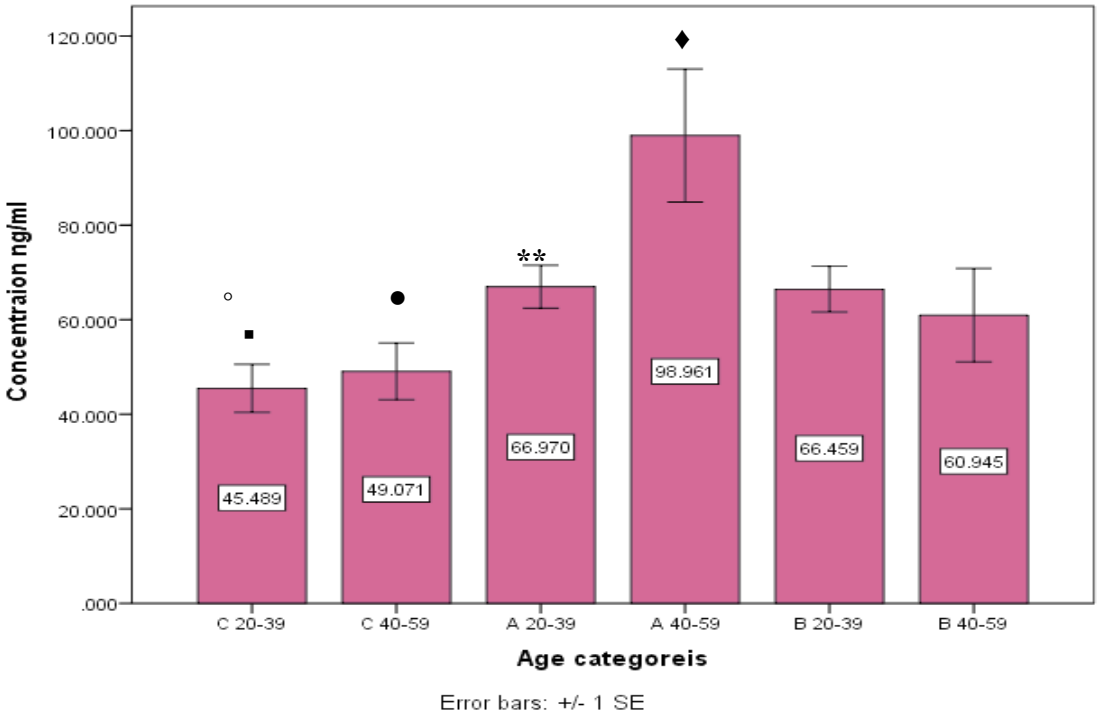


Figure 4-16: Concentration of IL-23 in blood sample of age categories of control group, patients group A and patients group B. The significance value was indicated as ◦ between 20-39 category of control group and 20-39 category of patients group A, ▪ between 20-39 category of control group and 20-39 category of patients group B, and● between 40-59 category of control group and 40-59 category of patients group A, and *between 20-39 category of patients group A and 40-59 category of patients group A and ♦ between 40-59 category of patients group A and 40-59 category of patients group B (the level of probability was indicated as ◦, ▪, ●, *,*,P ≤ 0.05, ◦◦, ▪▪,●●,, **,P ≤ 0.01, ◦◦◦, ▪▪▪,●●●,***, ***, P ≤ 0.001 and ◦◦◦◦, ▪▪▪▪,●●●●,****, **** P ≤ 0.0001).**

The result of current study showed elevate in the level of IL-23 in patients group than that control group, this result agreed with study published by Salah Hegab *et al*., 2014. High serum levels of IL-23 have been shown in SLE patients (Wong *et al*., 2008; Puwipirom *et al*., 2010).

By activating pathogenic Th17 cells, IL-23 can encourage SLE disease severity, operation, and kidny damage. For the production of pathogenic Th17 cells, IL-23 plays an important role which synthesize interleukine-17 that induce IL-6 generation and TNF-α, while interleukine-17 recruits neutrophils to tissues involved in SLE pathogenesis (Nobee et al., 2016).

IL-23 is also an inflammatory factor closely linked to SLE, and studies have shown that it primarily acts on Th17 cells and facilitates the secretion of IL-17, and it is involved in SLE, thrombocytopenic purpura, leukemia and so on (Paradowska *et al*.,2016)

Due to the lack of IL-23R in human and mouse naive T cells, IL-23 does not specifically promote Th cell differentiation. IL-23 causes naive CD4 T cells to differentiate into Th17 cells closely associated with autoimmune disease development (Larosa *et al*., 2019).

IL-23 development is stimulated by their ligands (including LPS, peptidoglycan, CpG DNA, and Poly I: C) by activating toll-like receptors (TLRs). These interactions lead to increased expression of p40 and p19, thereby increasing the release of IL-23 (yannam *et al*., 2012).

According to the result of current study the level of IL-23 increased in patients with infection than patients without infection.

IL-23 was evolutionarily involved in immune response by rapidly recruiting neutrophils during acute infection. This cytokine is released by a

cell presenting antigen within a few hours of exposure to LPS and other microbial products. This contributes to the activation of IL-17, which promotes the production of downstream proinflammatory cytokines by stromal cells, endothelial cells and monocytes, including IL-1, IL-6, IL-8 and tumor necrosis factor-α (Mok *et al*., 2010).

The concentrations of serum IL-23 did not vary by patient age and sex or by steroid treatment (Du, 2014). This study was in contrast with result of current study that showed significant increase in concentration of IL-23among age range (20-39) in patients group than control group.

4.4.5.3. Serum Interleukin-10 (IL-10) Levels Measurement

To investigate the role of IL-10 in patients and control the serum level of this cytokine has been measured. The mean level of IL-10 was (1.25 ± 0.06) in patients group and (1.25 ± 0.04) in the control group (Table 4-18 and Figure 4-17)

Table 4-18: Concentration of IL-10 in blood sample of control group and patients group

Marker	Group	No.	%	Mean ± S.E.	t-test	D.F	P.
IL-10	Control group	20	33.33	1.25 ± 0.04	0.01	58	0.99
	Patients group	40	66.67	1.25 ±0.06			

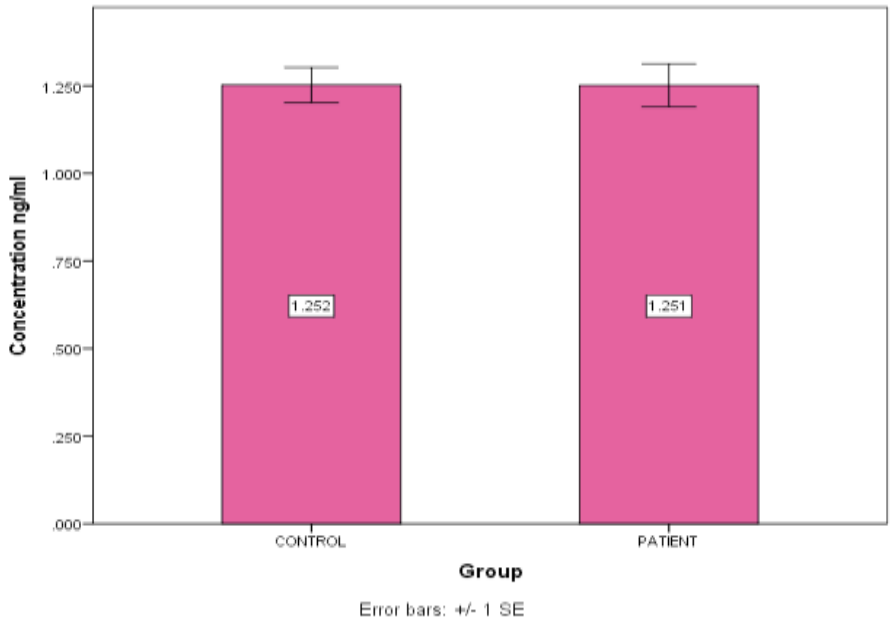


Figure 4-17-: Concentration of IL-10 in blood sample of control group, patients group The significance value was indicated as* between control group and patients group (the level of probability was indicated as * P ≤ 0.05, P ≤ 0.01,*** P ≤ 0.001 and **** P ≤ 0.0001).**

4.4.5.3.1. Serum Interleukin-10 (IL-10) Levels Measurement According To Groups

In the current study the level of IL-10 in patients group A, patients group B and control have been investigated. In patients group A mean level was (1.18 ± 0.10) and in patients group B was (1.28 ± 0.07). Thus the level of IL-10 in patients group B was higher than patients group A but did not significantly increase (Table 4-19, Figure 4-18).

Table 4-19 : Concentration of IL-10 in blood sample of control group, patients group A and patients group B

Marker	Comparisons Series	Group	No.	%	Mean ± S.E.	t-test	D.F.	P.
IL-10	1	Control group	20	33.33	1.25 ± 0.04	0.56	33	0.57
		Patients group A	15	25.00	1.18 ± 0.10			
	2	Control group	20	33.33	1.25 ± 0.04	0.39	43	0.69
		Patients group B	25	41.67	1.28 ±0.07			
	3	Patients group A	15	25.00	1.18 ± 0.10	0.78	38	0.43
		Patients group B	25	41.67	1.28 ±0.07			

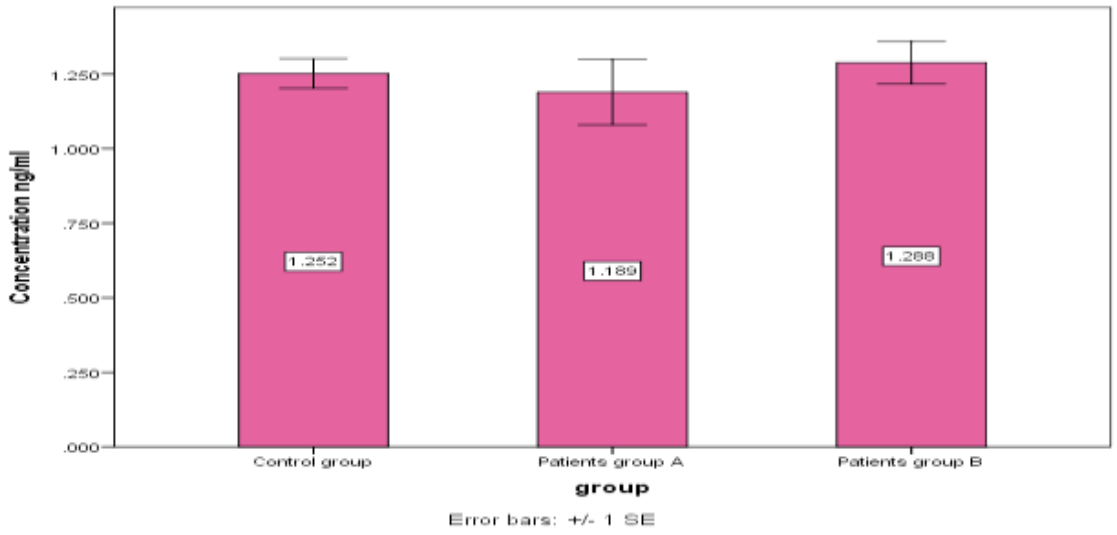
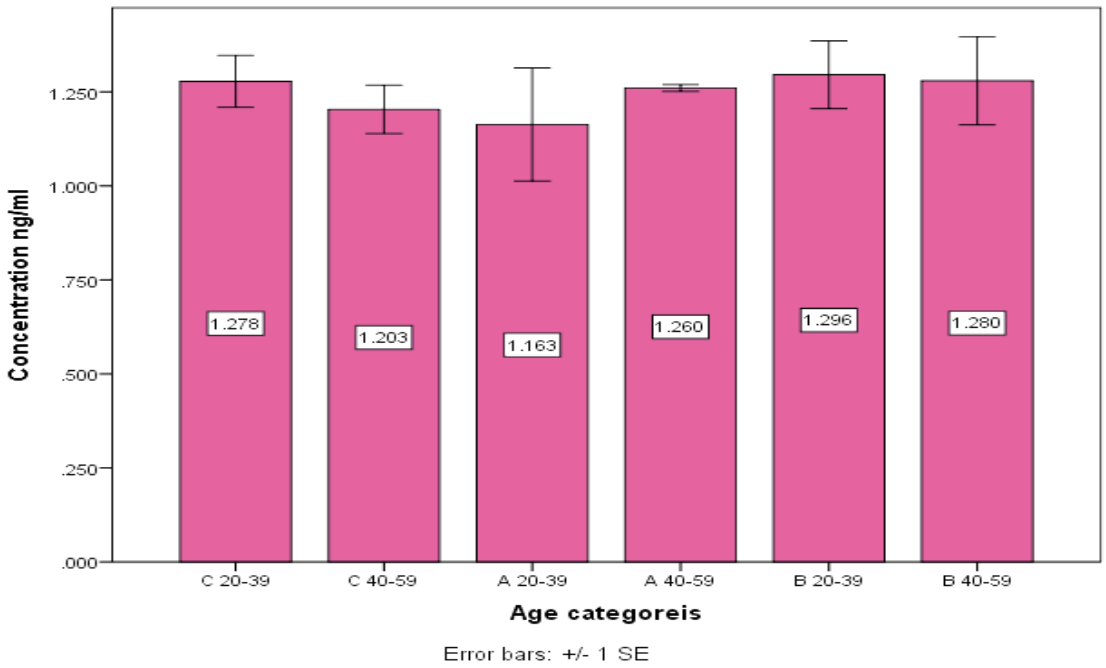


Figure (4-18); Concentration of IL-10 in blood sample of control group, patients Group A and patients Group B The significance value was indicated as * between control Group and patients Group A, ◦ between control group and patients Group B, (the level of the probability was defined as *, ◦ P ≤ 0.05, **, ◦◦ P ≤ 0.01, * , ◦◦◦ P ≤ 0.001 and **** , ◦◦◦◦ P ≤ 0.0001).**

4.4.5.3.2. Serum Interleukin-10 (IL-10) Levels Measurement According To Age Categories

By comparing between patients group and control group according to age the level of IL-10 has been investigated. The mean level of IL-10 among the age categories did not show significant differences among the age categories (Table 4-20 and Figure 4-19).



Figure(4-19): Concentration of IL-10 in blood sample of age categories of control group, patients group A and patients group B. The significance value was indicated as * between 20-39 category of control group and 20-39 category of patients group A, ▪ between 20-39 category of control group and 20-39 category of patients group B, and● between 40-59 category of control group and 40-59 category of patients group A, and ◦between 20-39 category of patients group A and 40-59 category of patients group A (the level of probability was indicated as ◦, ▪, ●, *,*,P ≤ 0.05, ◦◦, ▪▪,●●,, **,P ≤ 0.01, ◦◦◦, ▪▪▪,●●●,***, ***, P ≤ 0.001 and ◦◦◦◦, ▪▪▪▪,●●●●,****, **** P ≤ 0.0001).**

Table 4-20: Concentration of IL-10 in blood sample of age categories of control group, patients group A and patients group B

Marker	Comparisons Series	Group	No.	%	Mean ± S.E.	t-test	D.F.	P.
IL-10	1	Control group 20-39	13	21.67	1.27±0.06	0.70	18	0.49
		Control group 40-59	7	11.66	1.20 ±0.06			
	2	Patients group A 20-39	11	18.33	1.16 ± 0.15	0.37	13	0.71
		Patients group A 40-59	4	6.67	1.26 ± 0.00			
	3	Patients group B 20-39	13	21.67	1.29 ± 0.09	0.10	23	0.91
		Patients group B 40-59	12	20.00	1.27± 0.11			
	4	Control group 20-39	13	21.67	1.27±0.06	0.72	22	0.47
		Patients group A 20-39	11	18.33	1.16 ± 0.15			
	5	Control group 20-39	13	21.67	1.27±0.06	0.15	24	0.87
		Patients group B 20-39	13	21.67	1.29 ± 0.09			
	6	Patient group A 20-39	11	18.33	1.16 ± 0.15	0.78	22	0.44
		Patients group B 20-39	13	21.67	1.29 ± 0.09			
	7	Control group 40-59	7	11.66	1.20 ±0.06	0.64	9	0.53
		Patients group A 40-59	4	6.67	1.26 ± 0.00			
	8	Control group 40-59	7	11.66	1.20 ±0.06	0.46	17	0.64
		Patients group B 40-59	12	2.00	1.27± 0.11			
	9	Patients group A 40-59	4	6.67	1.26 ± 0.00	0.09	14	0.92
		Patients group B 40-59	12	20.00	1.27± 0.11			

In the current study no differences in serum IL-10 level in patients and control this result agreed with study published by (Dhir *et al*,.,2009) Such differences may have resulted from many possible causes,For example, size of sample, demographic different of patients, Clinical features or treatment kinds (Abd Elazeem et al., 2018).

IL-10 was observed to play a downregulatory function in early lupus, whereas in later stages of disease excessive development of IL-10 may lead to increased autoantibody production and the subsequent formation of pathogenic autoantibody – antigen complexes, As shown in *in vitro* studies, anti-IL-10 antibodies could significantly inhibit SLE peripheral blood mononuclear cells (PBMC) production of immunoglobulin, which confirmed IL-10's role in the mediation of autoantibody production (Yin *et al*., 2002).

IL-10 has been documented inhibiting nephritis, arthritis and neurological symptoms in an SLE mouse model. Low levels of IL-10 may therefore be correlated with active SLE. On the contrary, some studies indicate elevated IL-10 level in patients with SLE (Uchida *et al*., 2019).

IL-10 acts as a potent B cell stimulator which enhances B cell activation, proliferation and differentiation. This relates to SLE, which is characterized by high development of autoantibodies and reduced cellular immune responses. High levels of autoantibodies generate immune complexes in SLE which exacerbate tissue damage (Tournoy *et al*., 2000).

In general, IL-10 is known to be an anti-inflammatory and immunosuppressive cytokine. This is however overexpressed in lupus patients and in some cases it has been stated that it functions as a lupus promoting molecule. Increased levels of IL-10 were associated with disease activity or the development of autoantibodies which could contribute to SLE pathogenesis and/or modulate the differentiation and function of dendritic cells (Timóteo *et al*., 2016).

Aging is associated with decreases in adaptive and innate immunity, with elderly infections and autoimmune diseases occurring more frequently (Fuentes *et al*., 2017)

In the current study the results of CD4+Tcells and CD19+B cells level change with age, and we found significant difference in the level of these cells in SLE patients with infection compared to control between 20-39 and 40-59 years old, while when compared these cells in SLE patients with infection than SLE patients without infection we did not find significant difference in the level of CD4+Tcells in 20-39 compared to 40-59 age. While showed significant difference in 40-59 age range, The higher frequency of CD4+Tcells may explain their greater resistance to infectious diseases and greater probability of autoimmune disease development (Kokulna *et al*., 2019) but CD19+B cells showed significant difference between 20-39 and 40-59 age range.

Conclusion

1- Bacterial infections (UTI) in systematic lupus erythematosus patients have effect on the levels of CD4+T cells, CD19+ B cells.
2- CsgA gene in gram negative bacteria plays role in bacterial virulence.
3- There are pivotal differences in the levels of CD4+Tcells, CD19+B cells, HLA-DR, HLA-DQ and IL23 due to effect of infection and autoimmune disease (SLE).

Recommendations

1-Measurment the level of HLA-DR typing (HLA-DR2, HLA-DR3 and HLA-DR4) using flowcytometry, ELISA or PCR techniques.

2-Measurment the level of csgA protein in serum of patients by ELISA technique

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Appendix

Results Of Vitek2 System

Identification Information	Card: GN	Lot Number: 2410655103	Expires: Sep 12,2019 13:00
	Completed: Sep 10,2019 13:16 CDT		
Organism Origin	VITEK 2		
Selected Organism	99% Probability Escherichia coli Bionumber: 0405610550026611 Confidence: Excellent Identification		
SRF Organism			
Analysis Organisms and Tests to Separate:			
Analysis Messages:			
Contraindicating Typical Biopattern(s)			

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

Selected Organism: Klebsiella pneumoniae ssp pneumoniae

Identification Information	Card: GN	Lot Number: 2410655103	Expires: Sep 12,2019 13:00 CDT
	Completed: Sep 10,2019 13:16 CDT		
Organism Origin	VITEK 2		
Selected Organism	99% Probability Klebsiella pneumoniae ssp pneumoniae Bionumber: 6607734773564010 Confidence: Excellent Identification		
SRF Organism			
Analysis Organisms and Tests to Separate:			
Analysis Messages:			
Contraindicating Typical Biopattern(s)			

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

Selected Organism: Raoultella ornithinolytica

Identification Information	Card: GN	Lot Number: 2410655103	Expires: Sep 12,2019 13:00 CDT
	Completed: Sep 10,2019 13:16 CDT		
Organism Origin	VITEK 2		
Selected Organism	91% Probability Raoultella ornithinolytica Bionumber: 6637735777576010 Confidence: Good Identification		
SRF Organism			
Analysis Organisms and Tests to Separate:			
Analysis Messages:			
Contraindicating Typical Biopattern(s)			

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

Selected Organism : Pseudomonas Luteola

Identification Information	**Analysis time:** 5.35 hours	**Status:** Final
Selected Organism	96% Probability **Pseudomonas Luteola** **Bionumber:** 4001300150100212	
ID Analysis Messages		

Susceptibility Information			Analysis Time: 13.88 hours		Status: Final
Antimicrobial	MIC	nterpretation	Antimicrobial	MIC	Interpretation
ESBL			Imipenem	>=16	R
Ampicillin			Amikacin	>=64	R
Ampicillin/Sulbactam			Gentamicin	2	S
Piperacillin/Tazobactam	64	I	Tobramycin	4	S
Cefazolin	>=64	R	Ciprofloxacin	>=4	R
Ceftazidime	32	R	Levofloxacin	>=8	R
Ceftriaxone	>=64	R	Nitrofurantoin		
Cefepime	32	R	Trimethoprim/Sulfamethoxazole	<=20	S
Ertapenem					

Selected Organism : Staphylococcus haemolyticus

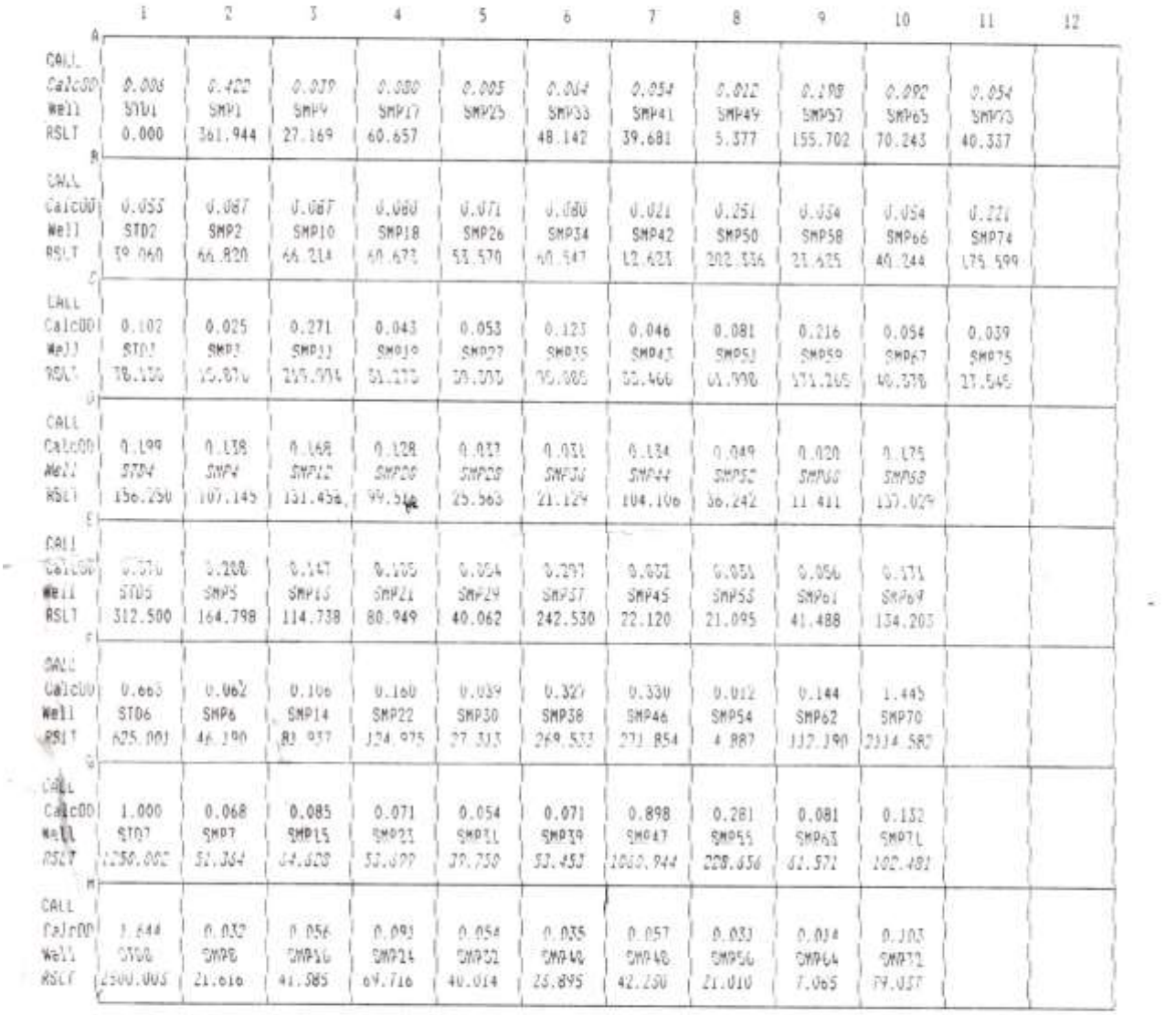
Identification Information	**Analysis time:**	8.00 hours	**Status:**	Final
Selected Organism	87% Probability	**Staphylococcus haemolyticus**		
	Bionumber:	010022103620271		
ID Analysis Messages				

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	-															

IL10 Data

	1	2	3	4	5	6	7	8	9	10	11	12
A	CALL Calc0D Well RSLT	0.021 STD1 0.000	0.040 SMP1 2.418	0.013 SMP9	0.020 SMP17	0.012 SMP25	0.010 SMP33	0.022 SMP41 0.233	0.022 SMP49 0.166	0.021 SMP49 0.011	0.013 SMP57	0.021 SMP73 0.046
B	CALL Calc0D Well RSLT	0.085 STD2 7.810	0.010 SMP2	0.013 SMP10	0.037 SMP18	0.016 SMP26	0.010 SMP34	0.055 SMP42 1.536	0.044 SMP50 2.971	0.022 SMP58 0.231	0.088 SMP88 8.478	0.012 SMP74
C	CALL Calc0D Well RSLT	0.148 STD3 15.630	0.012 SMP3	0.055 SMP11 4.274	0.013 SMP19	0.014 SMP27	0.045 SMP35 3.111	0.014 SMP43	0.020 SMP51	0.016 SMP59	0.081 SMP67 7.611	0.209 SMP75 24.086
D	CALL Calc0D Well RSLT	0.260 STD4 31.250	0.034 SMP4 1.674	0.038 SMP12 2.231	0.034 SMP20 1.712	0.008 SMP27	0.011 SMP36	0.011 SMP44	0.015 SMP52	0.010 SMP60	0.018 SMP68	0.017 SMP76
E	CALL Calc0D Well RSLT	0.450 STD5 62.500	0.010 SMP5	0.070 SMP13 6.196	0.006 SMP21	0.012 SMP29	0.104 SMP37 10.331	0.033 SMP45 1.588	0.029 SMP53 1.082	0.009 SMP61	0.025 SMP69 0.500	0.023 SMP77 0.303
F	CALL Calc0D Well RSLT	0.959 STD6 125.000	0.030 SMP6 1.222	0.012 SMP14	0.031 SMP22 1.287	0.012 SMP30	0.012 SMP38	0.021 SMP46 0.019	0.020 SMP54	0.019 SMP62	0.021 SMP70 0.068	0.017 SMP87
G	CALL Calc0D Well RSLT	1.439 STD7 250.000	0.027 SMP7 0.857	0.013 SMP15	0.009 SMP23	0.022 SMP31 0.167	0.092 SMP39 3.386	0.031 SMP47 1.314	0.064 SMP55 5.507	0.074 SMP63 6.768	0.180 SMP71 20.102	0.015 SMP79
H	CALL Calc0D Well RSLT	2.123 STD8 500.000	0.007 SMP8	0.013 SMP16	0.022 SMP24 0.171	0.011 SMP32	0.012 SMP40	0.014 SMP48	0.023 SMP56 0.248	0.010 SMP64	0.016 SMP72	0.023 SMP80 0.362

IL23 Data



	1	2	3	4	5	6	7	8	9	10	11	12
A												
CALL												
CalcOD	0.008	0.422	0.039	0.080	0.005	0.004	0.054	0.012	0.198	0.092	0.054	
Well	STD1	SMP1	SMP9	SMP17	SMP25	SMP33	SMP41	SMP49	SMP57	SMP65	SMP73	
RSLT	0.000	361.944	27.169	60.657		48.142	39.681	5.377	155.702	70.243	40.337	
B												
CALL												
CalcOD	0.055	0.087	0.087	0.080	0.071	0.080	0.021	0.251	0.054	0.054	0.221	
Well	STD2	SMP2	SMP10	SMP18	SMP26	SMP34	SMP42	SMP50	SMP58	SMP66	SMP74	
RSLT	19.060	66.820	66.214	60.672	53.570	60.547	12.623	202.336	21.625	40.244	175.599	
C												
CALL												
CalcOD	0.102	0.025	0.271	0.043	0.053	0.123	0.046	0.081	0.216	0.054	0.039	
Well	STD3	SMP3	SMP11	SMP19	SMP27	SMP35	SMP43	SMP51	SMP59	SMP67	SMP75	
RSLT	38.136	15.076	215.594	31.233	39.083	96.885	33.466	61.990	171.265	40.378	27.545	
D												
CALL												
CalcOD	0.199	0.138	0.168	0.128	0.037	0.031	0.134	0.049	0.020	0.175		
Well	STD4	SMP4	SMP12	SMP20	SMP28	SMP36	SMP44	SMP52	SMP60	SMP68		
RSLT	156.290	107.145	131.456	99.516	25.563	21.129	104.106	36.242	11.411	137.029		
E												
CALL												
CalcOD	0.376	0.208	0.147	0.105	0.054	0.297	0.032	0.031	0.056	0.171		
Well	STD5	SMP5	SMP13	SMP21	SMP29	SMP37	SMP45	SMP53	SMP61	SMP69		
RSLT	312.500	164.798	114.738	80.949	40.062	242.530	22.120	21.095	41.488	134.203		
F												
CALL												
CalcOD	0.663	0.062	0.106	0.160	0.039	0.327	0.330	0.012	0.144	1.445		
Well	STD6	SMP6	SMP14	SMP22	SMP30	SMP38	SMP46	SMP54	SMP62	SMP70		
RSLT	625.001	46.190	83.937	124.975	27.333	269.533	271.854	4.887	117.190	2314.582		
G												
CALL												
CalcOD	1.000	0.068	0.085	0.071	0.054	0.071	0.898	0.281	0.081	0.132		
Well	STD7	SMP7	SMP15	SMP23	SMP31	SMP39	SMP47	SMP55	SMP63	SMP71		
RSLT	1250.002	51.364	64.820	53.099	39.750	53.453	1060.944	228.656	61.571	102.481		
H												
CALL												
CalcOD	1.644	0.032	0.056	0.093	0.054	0.035	0.057	0.033	0.014	0.103		
Well	STD8	SMP8	SMP16	SMP24	SMP32	SMP40	SMP48	SMP56	SMP64	SMP72		
RSLT	2500.005	21.616	41.585	69.716	40.014	25.895	42.250	21.010	7.065	79.037		

الذئبة الحمراء الجهازية من امراض المناعة الذاتية الذي يؤثر على اعضاء وانسجة متعددة ويؤدي الى تلف هذه الانسجة وتلعب العوامل الوراثية والهرمونية والبيئية دورا في تطور المرض.

الدراسة الحالية اجريت في محافظة كربلاء.المجموع الكلي للعينات (60) عينة دم وادرار جمعت (40) عينة من النساء المصابات بالذئبة الحمراء الجهازية و(20) عينة من نساء اصحاء تتراوح اعمارهم (20-59) سنة. مجاميع المرضى قسمت الى مجموعتين بالاعتماد على نتائج الزرع البكتيري المجموعة الاولى تضم مرضى الذئبة المصابين بالبكتريا والمجموعة الثانية تضم المرضى غير المصابين بالبكتريا لتحديد تاثير الاصابة البكتيرية على مستوى (HLA-DQ,HLA-DR,CD19+Bcells,CD4+Tcells)في دم المرضى باستخدام تقنية flowcytometry وتحديد مستوى الانترلوكينات(10و23) في مصل المرضى باستخدام تقنية الاليزا واستخدمت تقنية تفاعل البلمرة التسلسلي لتحديد وجود الجين csgA في العينات البكتيرية .

الدراسة سجلت (15) اصابة بانواع مختلفة من البكتريا التي تصيب المسالك البولية في مرضى الذئبة الحمراء الجهازية حيث شخصت البكتريا باستخدام الفحوصات الكيميائية وجهاز الفايتك النتائج اظهرت زيادة معنوية في مستويات الخلايا (CD4+Tcells)في المرضى مقارنة بالاصحاء ($P\leq0.0001$) وكذلك زيادة معنوية في مستويات هذه الخلايا في المرضى المصابين بالبكتريا مقارنة بالمرضى غير المصابين واظهرت فروقا معنوية في مستوى الخلايا البائية في المرضى المصابين بالبكتريا مقارنة بالمرضى غيرالمصابين($P\leq 0.0001$) .

اما بالنسبة لمستوياتHLA-DR and HLA-DQ فاظهرت النتائج فروقا معنوية في مستوى HLA-DR في المرضى مقارنة بالاصحاء ($P\leq 0.01$) .

واظهرت نتائج الدراسة اختلاف معنوي في مستوى الانترلوكين23 في المرضى مقارنة بالاصحاء واختلاف معنوي في المرضى المصابين مقارنة بالاصحاء. ($P\leq 0.01$)

الاصابة البكتيرية في مرضى الذئبة الحمراء الجهازية المسببة لالتهاب المسالك البولية وخصوصا البكتريا csgA تؤدي الى تنشيط استجابة مناعية مكتسبة خصوصا الخلايا البائية والخلايا التائية.

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دورالاصابات البكتيرية في امراض المناعة الذاتية

رســالة مقدمــة إلـــى

مجلــس كليـة العلـوم ــ جامعـة كربلاء

وهـي جـزء مـن متطلبـات نيـل درجـة الماجستيـر علــوم فــي علــوم الحيــاة

من قبل الطالبة

ابتهال جواد ساجت

(بكالوريوس علوم / علوم الحياة –2016)

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