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Immunological Study in Systemic Lupus Erythoromatosus Patients Complaining from Chronic Renal Failure

A Thesis

Submitted to the Council of the College of Science at University of Kerbala in partial fulfillment of the requirements for the Master degree in Biology

By

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ ۚ
وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ﴾

صدق الله العلي العظيم

المجادلة 11

Dedication

To this who have reached the message… He advised the nation… To the Prophet of mercy and the light of the Worlds

(Prophet Muhammad peace be upon him)

To my wounded country …

(Iraq)

To the angel of my life… To the meaning of love and devotion… To whom her prayers was the secret of my success and a balm for my surgeon … To my love .

(Dear mother)

To whom Allah keep prestige and dignity… Who taught me to tender without waiting … To whom I bear his name proudly… To whom how supported and encouraged me .

(Dear father)

To my spiritual twin and companions … To sweet heart and honest intentions … To those who exist in my life earns me the power and infinite love.

(Dear Sisters)

I dedicate this work

Researcher
N. H. M.

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We the examining committee, certify that we have read this thesis entitle (**Immunological study in systemic lupus erythoromatosus patients complaining from chronic renal failure**)and examined the student (**Noor Hashim Al-Hashimi**) in its contents, and that according our opinion, it is acceptable as a thesis for the degree of Master of Science in Biology with (Excellent) estimation.

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Summary

Systemic lupus erythmatosus (SLE) is a chronic systemic autoimmune disease, marked serologically by both humoral and cellular immunologic abnormalities including multiple autoantibodies directed against non-organ specific selected intracellular antigens.

This study aimed to investigate some immunological markers which included (anti-nuclear antibodies, anti-double strand DNA) by used enzyme linked immune-sorbent assay (ELISA) technique, complement factor (C3, C4) by used nephelometry assay related to patients suffering from SLE. And their association with thymosin β4 level and vitamin D_3 concentration, in addition to the study of biochemical tests which included cystatin C, urea, uric acid and creatinine.

This study included sixty patients with systemic lupus erythematosus were attended to the Imam Hussein Medical City and thirty healthy control in Kerbala governorate during period from January 2015 to June 2015.

The majority of the SLE patients were female (95%) , the ratio of female male was 19:1. The mean age of SLE patients was (**36.11** ±1.53) year ranging from (7-65) compared with healthy control group, who matched in gender and age with patients.

Result's showed increased concentration of ANA and ds-DNA in patients with lupus nephritis in compared with mild and progressive form of SLE while serum complement factor (C3,C4) showed a significant decreased concentration in patients with lupus nephritis in compared with mild and progressive.

Furthermore, the result showed a significant decreasing in the level of thymosin β4 in lupus nephritis in compared with mild and progressive form of SLE.

The results also demonstrate that there was a significant decrease in vitamin D concentration in patients with lupus nephritis in compared with mild and progressive. On other hand this study showed that the treatment with vitamin D_3 supplement play important role as immunomodulate in patients with Systemic lupus erythmatosus.

Finally the results of the study also demonstrate that lupus nephritis in SLE patients has a high significant increasing in Cys-C concentration compared with mild and progressive.

The result can be calculate by using Cys-C index which included arrangement the SLE patients depended on the concentration of Cys-C.

Also, the result showed that Cys-C concentration was a highly significant in Cys-C index as compared with SLEDAI in SLE patients, and founded a more related result between Cys-C concentration, urea and creatinine concentrations in lupus nephritis.

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List of Abbreviations

Symbol	***Description***
Ab	Antibody
ACR	American College of Rheumatology
Ag	Antigen
ANA	Antinuclear Antibodies
Anti dsDNA	Anti-double stranded deoxyribosenucleus
C	Complement
CTD	Connective Tissue Diseases
Cys-C	Cystatin C
EBV	Epstein Barr Virus
ECLAM	European Consensus Lupus Activity Measurement
ESR	Erythrocytes Sedimentation Rate
GRF	Glomerular Filtration Rate
HLA	Human Leukocyte Antigen
HRP	Horseradish Peroxidase
IBD	Inflammatory Bowel Disease
IC	Immune Complexes
IDDM	Insulin Dependent Diabetes Mellitus
IL-2	Interleukin-2
INF-γ	Interferon Gamma
LN	Lupus Nephritis
LSD	Least Significant Difference
MS	Multiple Sclerosis
N	Number
NK	Natural Killer cells
OD	Optical Density
P	Probability

R	Correlation coefficient
R	Rang
RA	Rheumatoid Arthritis
RT	Room Temperature
SD	Stander Deviation
SE	Stander Error
SLE	Systemic Lupus Erythematosus
SLEDAI	Systemic Lupus Erythematosus Disease Activity Index
ss-DNA	Single strand deoxyribonuclease
Th	T helper cell
Thymosin β4	Thymosin beta 4
TMB	Tetramethyhybenzidine
TNF	Tumor Necrosis Factor
UV	Ultra Violet light

1.1: Introduction:

Systemic lupus erythematosus is an autoimmune inflammatory disease characterized by the presence of flare of autoantibodies, especially against nuclear components. Although it is believed that the etiology of SLE is multifactorial, including immune dysfunction, genetic, hormonal and environmental, the molecular mechanisms underlying this systemic autoimmune response remain largely unknown (Gangduo *et al.,* 2010).

SLE is known to be associated with polyclonal B cell hyper activity. Developing an understanding of the complex nature of human B cell differentiation (Dörner *et al.,* 2011).

Autoantibodies play an important role in the pathogenesis of SLE, and the diverse clinical manifestations of the disease are caused by the deposition of antibody-containing immune complexes in blood vessels, leading to inflammation in the kidney, brain, and skin. Direct pathogenic effects of the autoantibodies contribute to hemolytic anemia and thrombocytopenia (Graham *et al.,* 2007).

Antinuclear antibodies (ANA) positivity is usually considered as hallmark of SLE being positive in more than 95% of patients. Lupus nephritis has been associated with presence of many specific antibodies such as double strand deoxyribonucleic acid (ds-DNA) which correlates with the disease activity (Jain *et al.,* 2013).

Other intracellular organ antigen (Ag) associated with increasing in erythrocyte sedimentation rate (ESR) and deficiency of complement factor (C3, C4) dependent on systemic lupus erythematosus disease activity index (SLEAI) which related with kidney failure test to diagnoses lupus nephrite and using cystatin C test which specific marker to lupus nephritis, Adapted from (Bombardier *et al.,* 1992; Sdaile *et al.,* 1996).

Thymosin is a hormone related from thymus gland to labeled virgin T cell when can form bone marrow. There are two types of thymosin, alpha and beta thymosin. Alpha is labeling virgin T cell comes from the bone marrow and thymosin beta labeling virgin T cell in germinal sit in secondary immune organs, Thymosin β4 is detected outside of cells in blood plasma or in wound fluid, Several biological effects are attributed to thymosin β4, like induction of metallo-proteinases, chemotaxis, angiogenesis and inhibition of inflammation as well as the inhibition of bone marrow stem cell proliferation (Huff *et al.,* 2001).

Vitamin D_3 is the common denomination of a group of sterols with a crucial role in phospho-calcic metabolism. The main source of vitamin D_3 is the conversion of 7-dehydrocholesterol to pre-vitamin D_3 in the skin, by means of solar ultraviolet B radiation. Conversion to vitamin D_3, or cholecalciferol, also takes place in the skin through a heat-mediated process (Holick, 2007).

The major function of 1, 25(OH)2 D_3 is that it increases the absorption of calcium and phosphate from the intestinal tract, inhibits the secretion of parathyroid hormone (PTH), the proliferation of the parathyroid glands, endocrine system, proliferation of skin keratinocytes and importantly plays a significant role in the regulation of the immune system, therefore positively regulates bone formation (Anderson *et al.,* 2004; Morris, 2004 Baldock *et al.,* 2006).

Vitamin D_3 appears to act as an immunomodulator through its actions on the regulation and differentiation of immune cells like lymphocytes, macrophages, and natural killer cells (NK), besides interfering with the production of cytokines. Among the immunomodulatory effects demonstrated :inhibition of the production of autoantibodies by B lymphocytes and a reduction in the secretion of interleukin-2 (IL-2), gamma

interferon (INFγ), and tumor necrosis factor (TNF); inhibition of the secretion of IL-6 (Lemire *et al,*. 1992; Linker-Israeli *et al,*. 2001).

Cys-C is a low-molecular mass protein that is filtered freely through the glomerular membrane. Unlike creatinine, Cys-C is eliminated almost exclusively from the circulation by the kidney and is affected less by renal tubular secretion, theoretically making it an ideal marker of glomerular filtration rate (GFR) (Filler *et al.*, 2005).

meta-analysis suggested raw Cys-C concentration was a better indicator of kidney function than raw Creatinine concentration (Dharnidharka *et al.,* 2002).

Cys-C is produced by most cells of human body. It is a good marker of (GFR) because it is filtered at the glomerulus level freely and then reabsorbed and catabolized in the proximal renal tubules. In contrast to serum creatinine concentrations, Cys-C is not affected by gender and muscle mass, and therefore provides an accurate measure of renal function (Chew *et al.,* 2008).

It is particularly useful for early detection of renal impairment, a frequent complication of SLE (Waldman and Appel, 2006).

This test Cys-C could be beneficial to assess renal function in patients with lupus nephritis (LN) by which various factors associated with both disease (lupus) and the drugs used could modify the concentration of Cys-C (Kazama, *et al,*. 2002).

1.2: The Aim of Study:

This study aim to investigate some immunological and serological parameters in immune-disregulation in SLE disease.

These parameter included :

1- Detection of SLE disease by using SLEDAI and diagnosis test including (ANA, ds-DNA, complement C3, C4, and ESR).

2- Thymosin β4 as a hormone having anti-inflammatory properties in SLE patients.

3- Vitamin D_3 as an immunomodulater in SLE patients.

4- Correlation between the level of thymosin β4 and vitamin D_3 in SLE patient.

5- Correlation between the concentration of Cys-C and renal function tests including: (Urea, Uric acid, Creatinine) to evaluate the importance of them in early detection of impaired renal function in SLE patients.

2.1: Systemic Lupus Erythematosus (SLE):

The Systemic Lupus Erythematosus (SLE) is a systemic heterogeneous disorder characterized by a diverse clinical manifestations associated with B and T cell hyperactivity, overproduction of autoantibodies and the deposition of antigen-antibody immune complexes in blood vessels, renal glomeruli and joints (Guerra *et al.*, 2012).

The SLE pathogenesis results from various immune abnormalities involving impaired clearance of apoptotic cells and immune complexes (ICs) together with the reduction in the thresholds of activation of B and T lymphocytes, leading to loss of self-tolerance and overproduction of autoantibodies, which are mainly directed against DNA and RNA particles (Elkon and Santer, 2012; Belot and Cimaz, 2012).

The immune system can attack the body's cells and tissue in any part of the body, resulting in inflammation and tissue damage (James *et al.*, 2005).

It is a Type III hypersensitivity reaction caused by antibody-immune complex formation. The course of the disease is unpredictable, with periods of illness (called flares) alternating with remissions. The disease occurs nine times more often in women than in men, especially in women in child-bearing years ages 15 to 35, and is also more common in those of non-European descent (Rahman and Isenberg, 2008).

The causes are believed to be environmental, hormonal, or genetic factors, which results in a misdirected immune response in people who are genetically susceptible. Lupus is characterized by the presence of autoantibodies that are directed against a person's own proteins; these are most commonly anti-nuclear antibodies, which are found in nearly all cases.

These antibodies lead to inflammation (Lisnevskaia *et al.*, 2014; Bartels, 2015).

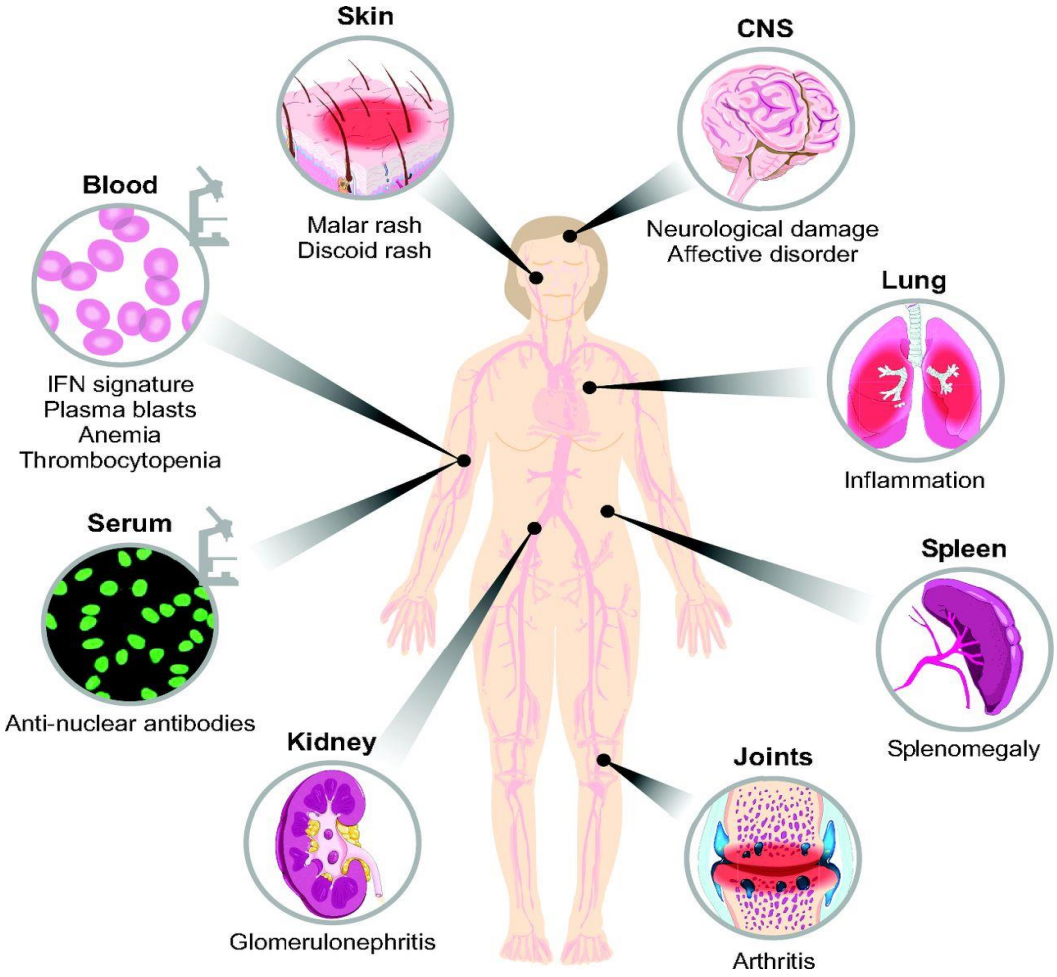


Figure 2. 1: The important symptoms in SLE patients.

2.2: The history of SLE:

The history of lupus erythematosus can be divided into three periods:

1- Classical

2- neoclassical

3- modern

The classical period: began when the disease was first recognized in the middle ages and saw the description of the dermatological manifestation of the disorder. Classical descriptions of the various dermatologic features of lupus were made by Thomas Bateman in the early nineteenth century;

Cazenave in the mid-nineteenth century; and Moriz Kaposi in the late nineteenth century. The first published illustrations of lupus erythematosus were included in von Hebra's text, Atlas of Skin Diseases, published in 1856 (Kaposi, 1872).

The Neoclassical period: of the history of lupus began in 1872 when Kaposi first described the systemic nature of the disorder. Kaposi proposed that there were two types of lupus erythematosus; the discoid form and a disseminated form. Over the last thirty years, pathological studies by Klemper and Baehr documented the structural changes in the glomeruli of lupus patients. Furthermore, Klemperer and colleagues in 1941, described SLE as one of the connective tissue diseases (CTD) according to observations at the autopsy table (Klemperer *et al.*, 1941).

The modern period began in 1948 with the discovery of the LE cell by Hargraves and coworkers. This period was characterized by advances in the knowledge of the pathophysiology and clinical-laboratory features of the disease, as well as advances in treatment (Hochberg, 1991).

In 1971, the American Rheumatism Association published preliminary criteria for the classification of SLE (Cohen *et al.*, 1971). Since then these preliminary criteria were revised and updated to incorporate new immunologic knowledge and improve disease classification. The 1982 revised criteria included fluorescence antinuclear antibody and antibody to native DNA and Sm antigen (Tan *et al.*, 1982). Due to the presence of anti-phospholipid antibodies and the anti-phospholipid syndrome was increasingly recognized in SLE patients, the Diagnostic and Therapeutic Criteria Committee of the American college of Rheumatology(ACR), updated the 1982 revised criteria for SLE in 1997 (Hochberg ,1997).

In Iraq, the first report concerning SLE was in 1971. There was a ten years follow up on 36 SLE cases on which clinical, laboratory and

pathological evaluations were carried out (AL-Damlujii *et al.*, 1971). In Iraq the SLE has been considered as the third most common inflammatory Rheumatic disease after rheumatoid arthritis and Rheumatic fever with incidence of 53/100000 of Iraqi population (Al-Rawi *et al.*, 1983).

Later on Iraqi researchers had become more interested about SLE and many studies had been made on different aspects of the disease (Al-Rawi *et al*, 1983; Hassan, 2000; sajid, 2002; Mohammad, 2007).

2.3: Epidemiology:

There is a worldwide prevalence of lupus ranges from 14 to 172 cases per 100,000 people .This wide variance is relative to racial, ethnic, and geographic factors (Wallace, 2008). The incidence increased three times in the last 40 years of the 20th century due to the improvement of the detection of early disease abnormalities (Uramoto *et al.*, 1999). Probable incidence rates are 1 to 25 per 100,000 in North America, South America, Europe and Asia (Rus *et al.*, 2002; Danchenko *et al.*, 2006).

2.4: Geographic and racial distribution:

- The prevalence of the disease is higher among Asian Indians compared with Caucasians in Great Britain, Afro-Americans, Afro-Caribbeans, and Hispanic Americans compared with Americans of European decent in the United States (Hochberg, 1985; Rus *et al.*, 2002). However, SLE occurs infrequently in Blacks in Africa (Symmons, 1995).
- The disease seems to be more common in urban than rural areas (Chakravarty *et al.*, 2007).

2.5: Etiopathogensis:

The lupus pathogenesis incorporates roles for genetic susceptibility based on a threshold model involving multiple genes; environmental

triggers, including microbial infection, sunlight, and certain drugs; and altered immune system function shows as in the figure (2.2). The individuals with clinical SLE usually experience over a period of many years, flares and remissions, associated with some organs damage related to chronic inflammation. Advance practical immunology has focused attention on the mechanisms that account for innate immune system activation. The genetic and environmental factors seem to play an important role in promotion of innate immune system activation and subsequent autoimmunity, while other aetiological factors may contribute to inflammation and tissue damage (Goldman & Ausiello, 2007).

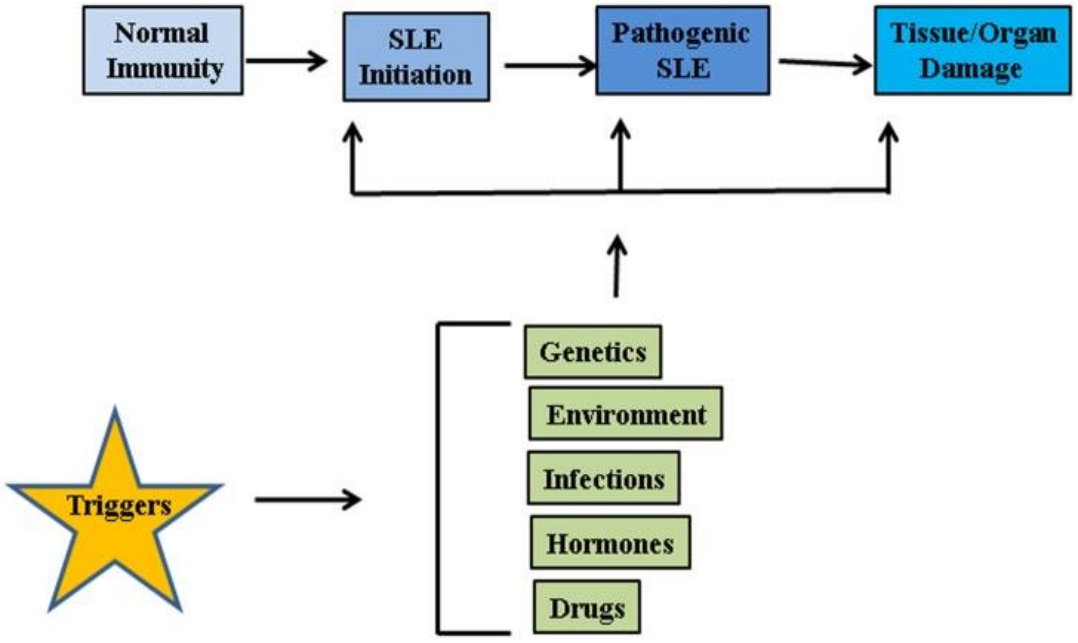


Figure 2.2: Factors associated with SLE pathogenesis. (Meena, 2013)

2.5.1: Genetic factors:

- There is no single lupus gene. It is a polygenic disorder. At least 30 susceptibility genes for SLE have been identified and their presence varies widely depending on race, ethnicity and geography (Croker & Kimberly, 2005).

- A genetic factor appears clearly from data obtained in first-degree relatives of SLE patients, the risk of SLE is about 20 times higher than in the general population. Also the rate in monozygotic twins range from 24 to 58% as compared with 3 to 10% for dizygotic twins (Hochberg, 1997).

- SLE association with the major histocompatibility antigen (HLA) were extensively investigated since 1971, many studies have shown that SLE is associated with HLA-DR3 and DR2 class II alleles, particularly in Caucasian individuals (Harley *et al.*, 1998).

- DR2 only association was reported in African Americans. However, these alleles carry a relatively small risk as compared with other autoimmune diseases (Walport & Morgan, 1991; Pickering, 2000).

2.5.2 The influence of the environment:

Many potential environmental triggers for lupus have been studied:

a) **Ultraviolet light exposure:** is a well-described trigger of lupus flares. Possible mechanisms that account for this observation include DNA damage and induction of apoptosis of keratinocytes, which results in concentration of nucleic acids and associated proteins in cell membrane blebs and increased availability of these self-antigens for processing by antigen-presenting cells. Exposure to ultraviolet light causes SLE flares in approximately 70% of patients (Goldman & Ausiello, 2007).

b) **The role of infections:** a role for microbial triggers, particularly virus infection has been postulated for many years, a viral theory as the etiologic agent had been adopted in 1952 by Mohan and Clark; however, they failed to prove it. An extensive investigation of the possible viral etiology was initiated in 1969 with the recognition of 2 viral-like structures in renal biopsies of SLE patients, which was

interpreted to be possibly a paramyxovirus nucleocapsid. However, they failed to fulfill the morophological criteria for these viruses (Klippel *et al.*, 1979). Epstein Bar virus (EBV) with Sm antigen among SLE patients (Sabbastini *et al.,* 1993).

It has been observed that children and adults with SLE are more likely to be infected by Epstein - Barr virus than age, gender and ethnically matched controls without SLE is intriguing, because EBV activates B lymphocytes and also contains amino acid sequences that mimic sequences on human spliceosomes common autoantibody specificity in people with SLE (Kasper *et al.*, 2005).

Moreover human infection with parvovirus B19, a small ss-DNA virus was reported to be associated with a variety of clinical manifestations in addition to elevated level of ANA, anti DNA, anti-lymphocyte and anti-cardiolipin antibodies (Finkel *et al.*, 1994; Nesher *et al.*, 1995).

c) **The role of drugs:** Pharmaceuticals have multifaceted actions ranging from exacerbating or aggravating an immune process to inducing lupus. For example, a drug can alter DNA or render it immunogenic and lead to the production of autoantibodies (e.g., procainamide). Other agents promote autoreactive T or B-lymphocytes (e.g., phenytoin). Hypomethylation of DNA results in altered DNA repair and autoantibody formation (e.g.,fludarabine). Oxidized metabolites of certain agents in slow acetylators, for example, can induce an immune reaction (e.g., hydralazine, isonaizid). Certain drugs are sun-sensitizing (e.g., nonarylamine sulfa antibiotics, phenothiazines) and lead to a phototoxic inflammatory response (Wallace, 2008).

A lupus-like syndrome has been seen in patients with hematologic malignancies or hepatitis C infection who are receiving doses of recombinant interferon-α. In addition, immunotherapy with anti-TNF agents has induced lupus autoantibodies and occasionally clinical lupus in patients with rheumatoid arthritis (Goldman & Ausiello, 2007).

d) The influence of hormone: Several Studies dealing with SLE denoted the effect of sex hormones on the occurrence of this disease with increased incidence in female more than male which may reach to fifteen fold (Hochberg, 1985).

Normally, women tend to mount more TH1 like responses than TH2 responses. During pregnancy, some women showed increased TH2-like responses. It is believed that pregnancy-associated levels of sex steroid may promote an anti- inflammatory environment. As a result diseases like SLE associated with high TH2-like responses, can be exacerbated during pregnancy, while diseases that involve inflammatory responses, such as rheumatoid arthritis, sometimes are ameliorated in pregnant women (Abd, 2010).

- The sex steroids that are circulating throughout the body can alter the immune responses by altering patterns of gene expression. Thus, estrogen enters cells, binds to the estrogen receptor, and thus induces the binding of the estrogen receptor to a specific DNA sequence, which in turn results in the modulation of transcription (Goldspy *et al*., 2000).

- Another hormone that may have a profound influence on Immune response in SLE patients is prolactin; which is expressed in high level in women than in men. Recent studies showed that the prevalence of anti-ds DNA, anti Sm, anti Ro and other antibodies have increased in

women with elevated level of prolactin hormone compared with women having normal prolactin level (Buskila *et al.,* 1995).

e) Toxic substances: a recently identified toxic exposure that increases risk of SLE is silica (Parks *et al.,* 2002). Smoking is a risk factor for SLE and, in addition, in patients with established SLE, smoking increases the risk of discoid lupus. Hair dyes, Pesticides and Solvents have been also identified to have some risk for SLE (Costenbader *et al.,* 2004).

2.6: Diagnosis:

The diagnosis of SLE is based on clinical and laboratory criteria. The criteria set developed by the American College of Rheumatology (ACR) are most widely used (Tan *et al.,* 1982; Hochberg 1997).

2.6.1 Autoantibodies

2.6.1.1 Anti-Nuclear Antibody (ANA)

Patients with SLE show positive ANAs test in about 95% of them using immunofluorescence screening, especially early in the disease, the sensitivity of ANA testing can be as low as 70% (Sjowall, *et al.,* 2008).

This fact is especially true in laboratories that are using enzyme immunoassays or other automated assays, which display marked inter-manufacturer variation in performance and have a reported sensitivity of 70–98% for the detection of ANA (Meroni & Schur, 2010).

Many individuals with positive ANA do not have SLE and have a low risk of developing the disease. So that, ANA screening has a low specificity for SLE, which lowers its use for diagnosis.

2.6.1. 2 Anti-double strand deoxyribonucleic acid (Anti-dsDNA)

Antibodies against double-stranded DNA which can be detected by the Farr assay, *Crithidia luciliae* immunofluorescence test (CLIFT) or

ELISA—are found in up to 70% of patients with SLE at some point during the course of their disease and have 95% specificity in established SLE cohorts, making them a valuable diagnostic marker (Craig and Ledue 2011).

The ds-DNA is always the principle antigen for anti-dsDNA antibodies in SLE. First described in the late 1950s autoantibodies to deoxyribonucleic acid (DNA) are highly heterogeneous with respect to their avidity, immunoglobulin subclass composition, cross-reactivity and complement fixing ability. In SLE, anti-dsDNA antibodies are classified according to their reactivity to ds-DNA; antibodies to single stranded DNA (ss-DNA) are not specific for SLE as they are found in sera from patients with both rheumatic and non-rheumatic diseases. (Feletar, 2003)

The prevalence of patients with SLE and a positive anti-DNA assay despite a negative ANA result has been reported to be 0–5.5% (Craig and Ledue 2011).

The risk of developing SLE or any other connective tissue disease within 5 years was not related to the CLIFT results. Together, these data emphasize that anti-DNA antibody testing should be performed only when there is reasonable suspicion for underlying connective tissue disease or SLE in ANA-positive individuals (George *et al.*, 2013).

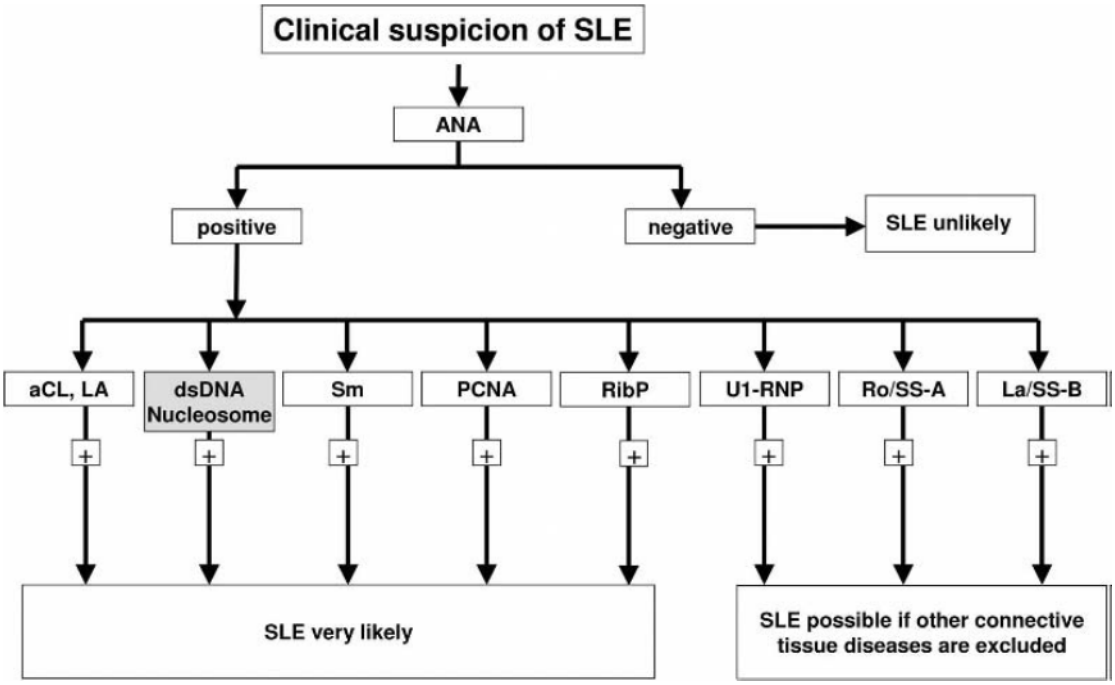


Figure 2. 3: Decision tree of autoantibody testing in patients with clinical suspicion of SLE (Pollard, 2006).

2.6.2 Serum complement

Acute exacerbations of the disease often are associated with decreased levels of serum complement. Serial measurements of C3 and C4 are routinely measured clinically, while testing for total hemolytic activity (i.e., CH50: Functional assays measure complement-mediated hemolytic activity, indicative of the activity of the classical pathway) or C1q is sometimes used to assess lupus disease activity (Liu and Ahearn, 2007).

2.6.3 Hematological finding:

The complete blood count screens for anemia, autoimmune hemolytic anemia, leucopenia, lymphopenia, and thrombocytopenia (Wallace, 2008).

The erythrocyte sedimentation rate (ESR), though a very nonspecific indicator of systemic inflammation is often monitored and in many patients can provide an indication of disease activity. Interestingly, C-reactive protein, an acute phase reactant, is relatively uninformative in SLE because it is often low in comparison to an erythrocyte sedimentation rate performed

on the same occasion (Goldman & Ausiello, 2007), except in the presence of serositis , and infection (Colledge *et al.*, 2010).

2.7: Causes of SLE

There are four main types of lupus:

1- Systemic lupus erythmatosus (SLE): the generalised and most common form.

2- Discoid Lupus erythmatosus: only affecting the skin.

3- Drug-induced Lupus: lupus caused by drugs.

4- Neonatal Lupus: Lupus in babies born to mothers with SLE. Systemic lupus erythematosus is the most common and serious form (William *et al.*, 2005; Rapini *et al.*, 2007).

2.8: Pathogenesis of tissue injure in SLE

Although knowledge of the etiology of SLE is incomplete, it is clear from the varied forms of tissue injury that a number of different effector mechanisms may act alone or in concert to produce the pleomorphic patterns as showen in figure (2.4) (Clynes *et al.*, 1998).

As well as by direct cytotoxicity, which is usually complement dependent, has been shown for antibody-mediated hemolytic anemia or thrombocytopenia.

In the kidney, intrinsic antigens such as extracellular matrix components or cell surface glycoproteins may serve as targets for autoantibody binding. In addition, renal injury in lupus nephritis may result from autoantibodies that bind to circulating antigens, forming circulating preformed immune complexes, or autoantibodies that bind to antigens deposited from the circulation in glomerular and vessel walls, causing in situ immune complex

formation, as has been shown for nucleosomes and antidouble-stranded DNA autoantibodies (Berden, 1997).

Subsequent Fc receptor and complement binding then initiates an inflammatory and cytotoxic reaction. Such cytotoxicity may be directed toward podocytes in the setting of membranous nephropathy, where in situ immune complex formation occurs along the sub epithelial aspect of the glomerular basement membrane, or toward endo capillary cells in the case of the endocapillary proliferative and exudative inflammatory reaction that follows subendothelial immune complex formation. In addition to direct immune complex-mediated cell and tissue injury, autoantibodies with antiphospholipid activity may also promote thrombotic and inflammatory vascular lesions in SLE (Daugas *et al.*, 2002).

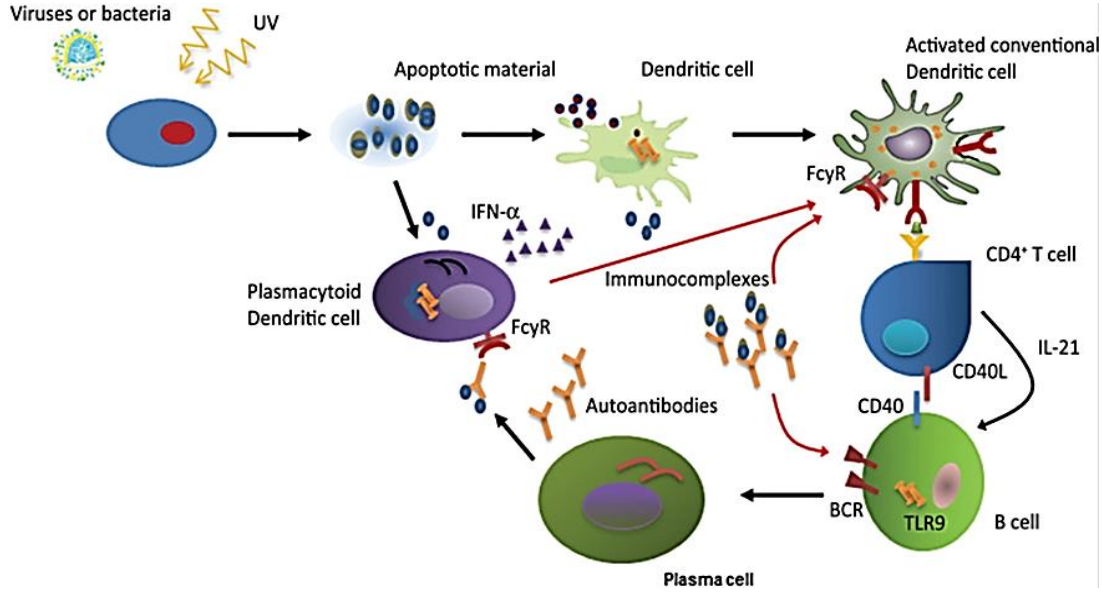


Figure 2. 4: Pathophsiology mechanism of SLE disease development. Adapted from (Bertsias *et al.*, 2010).

2.9: The complement system

The complement system represents the most important humoral effector branch of the immune defense. The system consists of at least 30 proteins circulating or membrane bound whose interactions enable the controlled activation of the cascade, the most important effects of complement

activation are: 1) membrane attack complex mediated direct lyses of target cells,(immune complex clearness) 2) opsonization, 3) initiation of inflammation by anaphylatoxins, 4) regulation of the cellular immune response (Müller-Eberhard, 1988).

Complement is activated by three different pathways: classical, lectin and alternative. All three shares the common step of activating the central component C3, but they differ according to the nature of recognition, the classical pathway was the first studied; it is activated by antibody released after a humoral response or by natural antibody (Reid and Porter, 1981).

2.9.1: Complement activation in SLE

In patients with SLE the complement is strongly activated, biopsies from inflamed tissues from patients with SLE shows Deposits of C3, C4, and associated complement proteins. Complement activity and classical pathway protein levels are associated with disease activity where they are reduced in active disease and increase following treatment. The initial cause of complement activation in SLE is thought to be the formation of high levels of immune complexes (IC) that, in turn, activate complement via the classical pathway, However, following the initial disease onset, there are a number of factors that may influence the degree of reduction of serum levels of complement components, these include disease activity parse, the rate of production versus catabolism, and importantly (Cameron *et al.*, 1976; Valentijn *et al.*, 1985).

There is a paradoxical association between complement and SLE, active SLE is accompanied by activation of the complement pathway, and it seems that complement participates in causing inflammatory damage to tissues in SLE. This pro-inflammatory role of complement in SLE is difficult to reconcile with the clinical observations, the inherited deficiency of classical pathway complement proteins plays an important role in the induction of

SLE. One hand, it provides important protective roles against the development of SLE. On the other hand, a direct pathogenic role for complement may still influence disease expression (Anthony *et al.*, 2003).

2.10: The Thymosin

The thymosins are a family of hormone like peptides originally isolated from the thymus gland, as initial as 1949, Abraham White, the father of modern thymic endocrinology, first suspected the vital role of the thymus gland in immune regulation, but it was not until the early 1960s that two groups of investigators, Goldstein and White in the USA, and Trainin in Israel, identified humoral factors with hormone-like activity from the thymus. Since that time over 20 different partially-purified thymic preparations have been described in the scientific literature and several unique peptides have been isolated and characterized.

Thymosin isolated from the thymus gland was first described in 1966 by Goldstein and White (Goldstein *et al.,* 1966). The β-thymosin family is composed of biochemically and functionally distinct polypeptides. Tβ4, Tβ10 and Tβ15, among the known family members, are found in humans (Goldstein *et al.*, 2005).

The most abundant peptide in this family, Tβ4, is a water-soluble and highly conserved, acidic polypeptide consisting of 43 amino acids and a molecular weight of 4964 Da (Low *et al.*, 1981).Tβ4 is selectively cross linked by factor XIIIa to various molecules, including collagen, fibrin and actin (Goldstein *et al.*, 2005).

It also acts as a major actin-sequestering molecule in all eukaryotic cells and is a potent regulator of actin polymerization in mammals (Sanders *et al.,* 1992). Emerging evidence proposes that Tβ4 is involved in a number of cellular responses, such as angiogenesis, hair growth, wound healing,

apoptosis, and inflammation (Philp *et al.,* 2004; Philp *et al.,* 2007; Sosne *et al.,* 2002)

Tβ4, released by platelets in the region of injured dermis, can accelerate dermal wound healing by stimulating cell migration, accelerating collagen deposition, and inhibiting both inflammation and apoptosis (Malinda *et al.,* 1999). Tβ4 stimulates the survival of cardiac myocytes after ischemia, an effect that is mediated by the increased expression of vascular endothelial growth factor (VEGF) and activation of integrin-linkedkinase (ILK) (Bock-Marquette *et al.,* 2004).

Recent studies have shown that Tβ4 is overexpressed in malignant tumors, and it has been suggested that it is associated with metastatic capability and angiogenesis (Larsson and Holck, 2007; Ji *et al,.* 2013).

Induced expression of Tβ4 has been shown to enhance tumor growth and metastasis in melanoma cell lines and mouse fibrosarcoma (Kobayashi *et al.,* 2002). In addition, Tβ4-overexpressing human colon cancer cells have exhibited increased growth and invasion in transplanted mice with those cancer cells (Wang *et al.,* 2004).

The mechanisms of the anti-inflammatory properties of Tβ4 remain poorly understood. Considering that Tβ4 is a major intracellular monomeric G-actin-sequestering molecule that also interacts with the focal adhesion proteins PINCH-1 and ILK, we investigated whether the anti-inflammatory properties of

Tβ4 are related to its association with actin and these intracellular binding partners. It's noted that Tβ4 inhibits TNF-α-mediated NF-κB activation, as well as the expression of the downstream proinflammatory gene IL-8 (Ping, 2011).

2.10.1: Tβ4 prevents apoptosis, promotes cell survival, and tissue regeneration

Tβ4 has been shown to diminish the proapoptotic effect of ethanol on human corneal epithelial cells in vitro by decreasing damaging mitochondrial alterations and cytochrome c release from mitochondria ، increasing bcl-2 expression and decreasing caspase activation (Sosne, 2004)

Tβ4 reduces apoptosis and induces survival genes. For example, it has been shown to inhibit endothelial apoptosis and activate the phosphoinositide /Akt cell survival signaling pathway in cardiomyocytes (Hinkel *et al.*, 2008).

It supports cardiac regeneration by inhibiting myocardial and endothelial cell death after infarction, inducing vessel growth and myocardial progenitor mobilization. Remarkably, Tβ4 is the only known molecule to initiate organ wide activation of the embryonic coronary development program in adult mammalian hearts (Bock-Marquette *et al.,* 2004; Hinkel *et al.,* 2008; Bock-Marquette *et al.,* 2009).

2.11: Vitamin D_3 concentration in SLE

Vitamin D_3, or cholecalciferol, is a lipid soluble hormone whose main action is the regulation of calcium metabolism, and bone formation and reabsorption through the interaction with the parathyroid glands, kidneys, and small bowel (Arnson *et al.,* 2007)

The classical, hormonal actions of vitamin D_3 are related to mineral metabolism and skeletal health. Vitamin D_3 increases intestinal calcium and phosphate absorption, stimulates osteoclast differentiation and calcium reabsorption from bone and promotes mineralization of the bone matrix. First evidence for the positive effect of vitamin D_3 intake for human health came from early studies on rickets and osteomalacia (Bouillon *et al.*, 2008).

2.11.1: Vitamin D Sources

Vitamin D may come from three potential sources: nutritional sources, UVB-dependent endogenous production and supplements. In humans, vitamin D is mostly synthesized in the skin after exposure to UVB whereas only a minor part is derived from dietary sources. Very few natural, non-fortified products such as fatty fish (mackerel, sardines, salmon ,cod liver oil) or some types of mushrooms (Shiitake), especially if sundried, contain relevant amounts of one of the two major forms, cholecalciferol (vitamin D_3) or ergocalciferol (vitamin D_2) (Holick, 2007; Lamberg-Allardt, 2006; Wacker and Holick, 2013).

Consequently, without supplementation, vitamin D status strongly depends on endogenous vitamin D production which is also influenced by genetic determinants, season, latitude, skin pigmentation and lifestyle such as the use of sunscreen and clothing (Wang *et al.*, 2010) .

2.11.2: Vitamin D Metabolism

In the human skin, cholecalciferol is synthesized from 7-dihydrocholesterol when exposed to UVB. Cholecalciferol is biologically inactive and immediately binds to vitamin D binding proteins or albumin. It then enters the circulation and is hydroxylated in the liver, catalyzed by the enzymes CYP2R1 and CYP27A1, which results in the production of the inactive form 25-hydroxyvitamin D-25(OH)D which represents the main circulating vitamin D metabolite and is the most reliable parameter to define human vitamin D status (Heaney, 2012).

In the kidney, 25(OH)D is further converted to the circulating biologically active compound cholecalciferol (1,25(OH)2D) by the enzyme 1-α-hydroxylase (CYP27B1) which is under strict control of parathyroid hormone and the phosphaturic hormone fibroblast growth factor 23, show as in the figure (2.5).

Their important role is not only in calcium metabolism and bone formation, but also their immune-modulatory action, which is not surprising, since vitamin D receptors are expressed in different tissues, such as brain, heart, skin, bowel, gonads, prostate, breasts, and immune cells, as well as bones, kidneys, and parathyroid glands (Jones and Twomey, 2008).

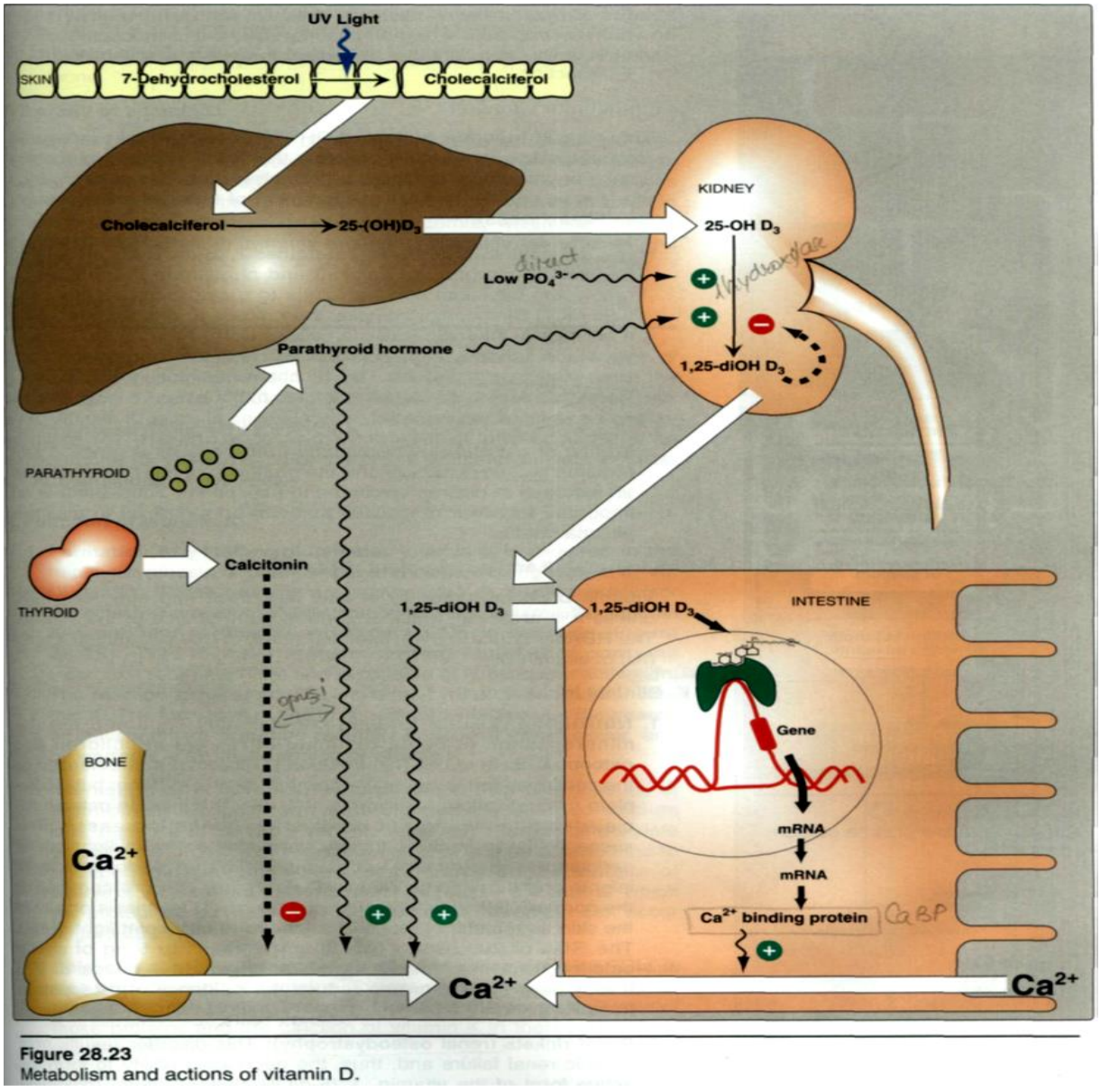


Figure 28.23
Metabolism and actions of vitamin D.

Figure 2. 5: Metabolisim and action of vitamin D. (Lippincott)

2.11.3: Vitamin D and The immune-regulatory role

Vitamin D, besides having well-known control functions of calcium and phosphorus metabolism, bone formation and mineralization, also has a role

in the maintenance of immune-homeostasis. The immune-regulatory role of vitamin D affects both the innate and adaptive immune system contributing to the immune-tolerance of self-structures. Impaired vitamin D supply regulation, amongst other factors, leads to the development of autoimmune processes in animal models of various autoimmune diseases. The administration of vitamin D in these animals leads to improvement of immune-mediated symptoms. Moreover, in human autoimmune diseases, such as multiple sclerosis, or rheumatoid arthritis the pathogenic role of vitamin D has been described.

Several studies correlated vitamin D deficiency with several autoimmune disorders, including insulin dependent diabetes mellitus (IDDM), multiple sclerosis (MS), inflammatory bowel disease (IBD), systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA)(Jones and Twomey, 2008; Cantorna, 2000; Kamen *et al*,. 2006; Lipps, 2004).

It is clearly apparent that Vitamin D_3 can interact with the immune system through its actions on the function and differentiation of cells like lymphocytes, macrophages, and natural killer cells (NK), in addition to interference with the in vivo and in vitro production of cytokines. Among the immunomodulatory effects demonstrated we should mention a reduction in the production of interleukin-2 (IL-2), gamma interferon (INFγ), and tumor necrosis factor (TNF); inhibition of the expression of IL-6; and inhibition of the secretion and production of autoantibodies by B lymphocytes (Lemire *et al.*, 1992; Linker-Israeli *et al.*, 2001).

2.11.4: Vitamin D_3 and autoimmune diseases:

The role of vitamin D_3 action on the immune system is demonstrated by enhancement of innate immunity associated with the regulation of acquired immunity (Adorini and Penna, 2008).

It has been suggested that vitamin D_3 and its analogues not only hamper the development of autoimmune diseases, but they can also be used as a treatment (Szodoray *et al.,* 2008).

Vitamin D_3 supplementation has been shown to be effective in management of various experimental animal models, such as allergic encephalomyelitis, type 1 diabetes mellitus, collagen-induced arthritis, inflammatory bowel disease, autoimmune thyroiditis, and SLE (Arnson *et al.,* 2007).

Several authors have been demonstrated that there was a higher prevalence of vitamin D_3 deficiency in SLE patients as compare with healthy individuals (Kamen *et al.,* 2006; Bultink *et al.,* 2005).

The concentration of vitamin D_3 were significantly lower in SLE patients (mean 13 ng /ml) as compared with RA, osteoarthritis and healthy control (24 ng/mL; 32 ng/mL; 27 ng/mL) respectively (Huisman *et al.,* 2001).

Also 50% of SLE patients had vitamin D_3 deficiency (cut off <50 nmol/L or 20 ng/mL). However, when those individuals were compared to those with fibromyalgia, differences in PTH, 25(OH) D, and 1.25(OH2)D_3 levels were not observed. Patients with systemic lupus erythematosus have multiple risk factors for 25 (OH)D deficiencies about the recommendation to apply sunscreen to avoid photosensitivity are responsible for lower sun exposure, decreasing the production of vitamin D in the skin. In addition to the Long term use of corticosteroids and hydroxichloroquine seems to affect vitamin D metabolism, although evidence are not so clear yet. On the other hand, severe renal involvements which can be seen in patients with lupus nephritis, can affect the hydroxylation step of 25(OH)D (Ruiz-Irastorza *et al.,* 2008).

Among the main functions of vitamin D in the immune system, we could mention activation of CD4 lymphocytes differentiation (Cantorna and

Mahon, 2004; Cutolo and Otsa, 2008). Increase in the number and function of regulatory T cells (Treg) (Szodoray *et al.,* 2008).

In vitro inhibition of the differentiation of monocytes in dendritic cells reduction in the production of cytokines, interferon-λ, TNF-α and IL-2 by Th1 cells, and stimulation of the function of Th2 helper cells (Cutolo, 2009; Arnson *et al.,* 2007).

A relationship between vitamin D deficiency and the prevalence of some autoimmune diseases like IDDM, RA, MS, SLE, and IBD has been demonstrated (Ruiz-Irastorza *et al.,* 2008).

The relationship between vitamin D deficiency and RA is based on two facts: evidence indicates that patients with RA have vitamin D deficiency and the presence of 1.25(OH)2D3 and VDR in macrophages, chondrocytes, and synovial cells in the joints of those patients (Nagpal and Na, 2005; Manolagas *et al.,* 1986).

Oral supplementation with high doses of cholecalciferol for three months reduced the severity of the symptoms in 89% of the patients, 44% had satisfactory results and 45% of them achieved complete remission. The incidence of side effects, like hypercalcemia, was observed in a low rate. Also it seems that there is an inverse relationship between disease activity and the concentration of vitamin D metabolites in patients with inflammatory arthritis. Also the critical concentration of vitamin D (<10 ng/mL) are more common in patients with renal involvement and photosensitive skin lesions (Kamen *et al.*, 2006).

According to the SLEDAI (Systemic Lupus Erythematosus Disease Activity Index) and ECLAM (European Consensus Lupus Activity Measurement) has been documented that The association between low serum concentration of vitamin D and disease activity scores (Cutolo and Otsa, 2008; Thudi *et al.,* 2008).

2.11.5: Toxicity of vitamin D

The most toxic of all vitamins is vitamin D. it is a fat-soluble vitamin, can be stored in the body and is only slowly metabolized, high doses of it (for weeks or months) can cause loss of appetite, nausea, thirst, and stupor hypercalcemia results from Enhanced calcium absorption and bone resorption, which can lead to deposition of calcium in many organs, particularly the arteries and kidneys.(Lippincott)

2.12: Cystatin C

Cystatin C (Cys-C) is a low molecular mass protein that was initially known as inter alia g-trace, post-g-globulin, and gamma-CSF. The amino acid sequence of the single polypeptide chain of human cys-C was determined in 1981 (Grubb and Lffberg, 1982).

Cys-C is unique among cystatins as it seems to be produced by all human nucleated cells. It is a good marker of glomerular filtration rate (GFR) as it is freely filtered at the glomerulus and then reabsorbed and catabolized in the proximal renal tubules. In contrast to serum creatinine concentrations (Chew, *et al.*, 2008).

It is particularly useful for the detection of mild renal impairment, a frequent complication of SLE (Waldman and Appel, 2006).

A series of studies have shown that Cys-C is not affected by factors such as muscle mass, age, gender, diet, inflammation or tumor (Nilsson Ehle and Grubb, 1994; Helin *et al.*, 1998).

2.12.1: Cys-C as a marker of GFR (glomerular filtration rate)

Small molecular weight proteins have long been proposed as markers of GFR as they are normally almost freely filtered through the normal glomerular membrane (Jung, 1987). In a normally functioning kidney, these

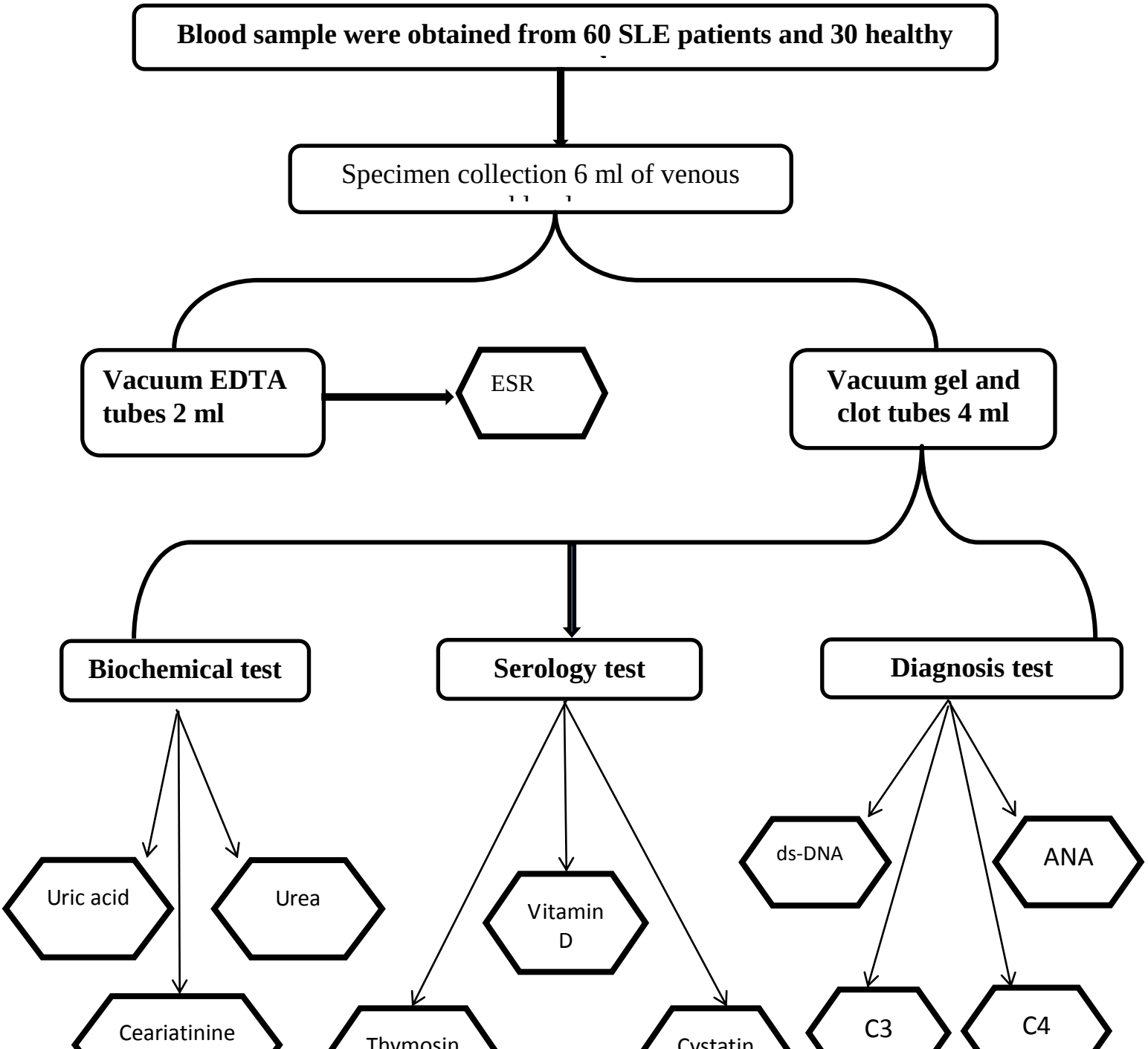
small molecular weight proteins should then be almost completely reabsorbed and degraded by proximal tubular cells. Really, studies of the handling of human cys-C in the rat have shown that the plasma renal clearance of Cys-C is 94% of the renal clearance of the generally used GFR marker (Grubb *et al.*, 1985).

Early investigations demonstrated that serum Cys-C was really a marker of GFR, at least as good as serum creatinine in the populations investigated (Simonsen *et al.*, 1985; Lffberg and Grubb, 1979).

These studies also showed that the serum Cys-C concentration was a better GFR marker than the serum levels of the other low molecular mass proteins investigated, such as h2-microglobulin, retinol binding protein, and complement factor D (Bfkenkamp *et al.*, 2002).

3. Materials and Methods

3.1 General Layout of the study



3.2 Materials:

3.2.1. Equipment's and Instruments:

The equipment's and instruments used in this study are described in the table (3.1)(3.2).

Table 3. 1: Equipment's .

NO	Tools	Company	Country
1	Centrifuge	Kokusan H-19F	Japan
2	Deep freez -70 C	Ateko	Denmark
3	ELISA Human	Human	Germany
4	Genius	PA54	China
5	Incubator	Memmert	Germany
6	Refrigerator	Concord	Lebanon
7	TOSOH	P 180	Japan
8	Vortex	Thermolyne	USA

Table 3. 2: Instruments.

NO	Tools	Company	Country
1	Cuvlte	Genius	China
2	Cylinders 250,500 ml	HBG	Germany
3	Disposable sterile blue, yellow and white tips		Germany
4	Disposable sterile syringe		Germany

5	Disposable tube	China	China
6	EDTA K3 tube 2 ml Vacuum	AFCO	Jordan
7	Eppendorff tube	Eppendorff	Germany
8	ESR racks	AFCO	Jordan
9	ESR tube	AFCO	Jordan
10	Micropipettes 0.5-10 µl / 10-100 µl /100-1000 µl	Fisher Scientific	U.S.A
11	Multichannel pipettes		
12	Racks for 50 tube	China	China
13	Vacuum Gel &Clot Active tube 6ml	AFCO	Jordan

3.2.2.Prepared Kits :

The commercial kits used in the study are shown in table (3.3).

Table 3. 3: commercial kits used in the study.

NO	Type of kits	Company	Country
1	25-OH vitamin D ELISA	Euroimmun	Germany
2	ANA screen	Generic assay	Germany
3	Anti-dsDNA-NcX ELISA(IgG)	Generic assay	Germany
4	Cearіatinin	Human	Germany
5	Complement C3	Genus	China
6	Complement C4	Genus	China
7	Cystatin C		Japan
8	Thymosin beta 4	Hcusaio	Germany
9	Urea	Randox	United Kingdom
10	Uric acid	Randox	United Kingdom

3.3 Methods :

3.3.1 Data collection

The study was conducted in the Imam Hussein Medical City in Karbala governorate during period from January 2015 to June 2015, during the first year of our work.

3.3.2: Classification Criteria

The classification criteria for SLE were sited 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 3.4: Eleven 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	headaches, seizures, amnesia, decreased ability to

disorder	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 (<100,000 platelets 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

3.3.3: Assessment of Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)

Several worldwide organ-specific activity indices are commonly used in the evaluation of SLE patients. These indices have been cited according to the long term observational studies and have been shown to be strong predictors of damage and mortality that reflects certain disease activity. Moreover, they have been validated against each other (Urowitz and Gladman, 1998).

Table 3.5: Several worldwide organ-specific activity indices are commonly used in the evaluation of SLE patients (Bombardier *et al.*, 1992: Sdaile *et al.*, 1996).

weight	Descriptor	Definition
8	Seizure	Of Recent onset. Exclude drug-related metabolic, or infectious causes
8	Psychosis	severe disturbance in the perception of reality cause Altered ability to function in normal activity. Includes hallucinations; non-coherence; marked loose associations; impoverished thought content;

		marked illogical thinking; bizarre, disorganized or catatonic behavior.
8	Organic brain syndrome	impaired memory due to altered mental function with impaired orientation or other intellectual function, with rapid onset and fluctuating clinical features. Includes a clouding of consciousness with a reduced capacity to focus and an inability to sustain attention on environment, and at least two of the following: perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, increased or decreased psychomotor activity. Exclude metabolic, infectious, and drug-related causes.
8	Visual	systemic lupus erythematosus result's in retinal changes which include cytoid bodies, retinal hemorrhages, serous exudates or hemorrhages in the choroid, optic neuritis (not due to hypertension, drugs, or infection).
8	Cranial nerve	sensory or motor neuropathy involving a cranial nerve.
8	Lupus headache	Severe, persistent headache; may be migranous; unresponsive to narcotics.
8	Cerebrovascular accident	New syndrome. Exclude arteriosclerosis.
8	Vasculitis	Ulceration, gangrene, tender finger nodules, periungual infarction, splinter hemorrhages. Vasculitis confirmed by biopsy or angiogram.
4	Arthritis	Involvement of More than 2 joints with pain and signs of inflammation.
4	Myositis	These include Proximal muscle aching or weakness associated with elevated creatine phosphokinase/aldolase levels, electromyographic changes, or a biopsy showing myositis.
4	Casts	Heme, granular, or erythrocyte
4	Hematuria	More than 5 erythrocytes per high power field. Exclude other causes (stone, infection).
4	Proteinuria	More than 0.5 grams of urinary protein excreted per 24h. New onset or recent increase of > 0.5 g/24h.
4	Pyuria	More than 5 leukocytes per high-power field.

		Exclude infection.
2	New malar rash	New onset or recurrence of an inflammatory type of rash.
2	Alopecia	New or recurrent. Apatch of abnormal, diffuse hair loss.
2	Mucous membranes	New onset or recurrence of oral or nasal ulcerations.
2	Pleurisy	Pleuritic chest pain with pleural rub or effusion, or pleural thickening.
2	Pericarditis	Pericardial pain with at least one of rub or effusion. Confirmation by electro- or echocardiography.
2	Low complement	Adecrease in CH50, C3, or C4 level (to less than the lower limit of the laboratory-determined normal range).
2	Increased DNA binding	More than 25% binding by Farr assay (to >the upper limit of the laboratory-determined normal range, e.g. 25%).
2	Fever	More than 38 ºC after the exclusion of infection.
2	Thrombocytopenia	Fewer than 100,000 platelets
2	Leukopenia	Leukocyte count of < 3000/mm3 (not due to drugs)

Total source	**0**	**1**	**2**	**3**
	None	**Mild**	**Med**	**Sever**

3.3.4 Patients a questionnaire survey

Patients a questionnaire survey

Sequence: ☐

hospital	
Full Name	
Age	
Gender	Male ☐ female ☐
home adress	
Telephone number	
Social status	
Main symptoms	joint pain ☐ molar rash ☐ photosensitivity ☐ Anemia ☐ Arthritis ☐ Hair Loss ☐ Oral ulcer ☐ renal failure ☐

chronic diseases	Pressure ☐ Heart Disease ☐ Sugar ☐ Kidney disease ☐ Diseases of the joints ☐ Fat content in the blood ☐
Medicine used	
History of the disease	

3.3.5 Patient and control

This study has been performed on 60 patients suffering from SLE including (3 males and 57 females). Their ages range was (7-65) years old and apparently healthy control 30 were selected to participate as a normal group for comparison with age group and sex matching of patients attending to the Imam Hussein Medical City in Karbala governorate during period from January 2015 to June 2015.

Then immunological parameter is measured such as diagnosis test ANA, ds-DNA, complement factor (C3, C4), thymosin β4, vitamin D_3, ESR and biochemical test is measured such as Cys-C, urea ,uric acid and cearitinin , in both patient and control groups. Data on the following variables are recorded SLEDAI.

3.3.6 Samples Collection:

Six milliliters of blood sample have been drawn from each patients and healthy persons by vein puncture using disposable syringes under aseptic technique.(Barenfanger *et al.*, 2004). Blood of each sample has been divided to two part. One milliliters is transferred into vacuum EDTA tubes for measuring ESR . The remaining five milliliters have been transferred into vacuum gel and clot tubes left at room temperature for at least 30 minutes

for clotting then centrifuge at 4000 rpm 4 minuts. Then separated serum has been divided into four Eppendorff tubes and stored at -70° C until used to avoid repeated thawing and freezing**.** For measuring immunological and serological test ANA, ds-DNA, Complement factor (C3, C4), Thymosin β4, Vitamin D_3 and biochemical test which included Cys-C, Urea, Uric acid and Creatinine.

3.3.7 Hematology Test

Specimens of venous blood about 2 ml in EDTA tube has been used to determine ESR.

3.3.7.1 Erythrocytes Sedimentation Rate (ESR)

Erythrocytes Sedimentation Rate was measured using Westergren method (Lewis & Bain 2001). Using disposable ESR tube containing 0.5 ml of sodium citrate add 0.2Ml blood and mix the sample thoroughly and then draw it up into Westergren-tube to the 200 mm mark by means of mechanical device. The tube is placed in a ESR rack in a strictly vertical position for 1 hour at room temperature, at which time the distance from the lowest point of the surface meniscus to the upper limit of the red cell sediment is measured the distance of fall of erythrocytes, expressed as millimeters in 1 hour.

3.3.8 Immunological Study:

3.3.8.1 Enzyme immunoassay for the determination of IgG antibodies to nuclear and cytoplasmic antigens in human serum and plasma (ANA screen):

3.3.8.1.1 Principle of the test

ANA screen is used for the semi-quantitative determination of autoantibodies to nuclear and cytoplasmic antigens in human serum and plasma.

Antibodies of the controls and diluted patient samples react with nuclear and cytoplasmic antigens immobilized on the solid phase of microtiter plates. Using complete HeLa nuclei enriched with recombinant and native antigens guarantees the specific binding of autoimmune antibodies of specimen under investigation. Following an incubation period of 60 min at room temperature (RT, 18-25 ºC), unbound sample components is removed by a wash step.

The bound IgG antibodies react specifically with anti-human-IgG conjugated to horseradish peroxidase (HRP). Within the incubation period of 30 min at RT, excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colorless substrate solution of 3, 3', 5, 5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution into the wells after 15 min at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound. Patient ratios are calculated by dividing the respective OD of the specimen with the calculated cut-off OD.

3.3.8.1.2 Assay procedure:

- Dilute patient sera with sample diluent (c) 1+100 (v/v),

  e.g. 10µl sample +1 ml sample diluent (c).

- Avoid any time shift during pipetting of reagents and sample.

1- Bring all reagent to room temperature (18-25 ºC) before use.

Mix gently without causing foam.

2- Dispense :

100µl controls P, CO, N

100µl diluted patient samples into the respective wells.

3- Cover plate, incubate 60 min at room temperature (18…25 ºC).

4- Decant, then wash each well three times using 300µl wash solution (made of B).

5- Add 100µl of conjugate (D) solution to each well.

6- Cover plate, incubate 30 min at room temperature (18…25 ºC).

7- Decant, then wash each well three times using 300µl wash solution (made of B).

8- Add 100µl of substrate (E) to each well.

9- Incubate 15 min protected from light at room temperature (18…25 ºC).

10- add 100µl of stop solution (F) to each well and mix gently.

11- Read the OD at 450 nm versus 620 or 690 nm within 30 min after adding the stop solution.

Anti-nuclear antibody was performed by indirect ELISA technique, for semi quantification of the results; each patient of optical density (OD) value can be expressed by the index-value which is calculated by dividing the patient-OD by the cut-off OD (1.0).

3.3.8.2 Anti-dsDNA-NcX ELISA (IgG) test instruction

3.3.8.2.1 Principles of the test

Anti-dsDNA is an enzyme immunoassay for the quantitative determination of lgG antibodies to dsDNA .The antibodies of the calibrators, control and diluted patient samples react with dsDNA immobilized on the solid phase of microtiter plates. Highly purified ds-DNA coated on the microtiter plate guarantees the specific binding of dsDNA lgG antibodies of

the specimen under investigation. Following an incubation period of 60 min at room temperature (RT), unbound sample components are removed by a wash step.

The bound IgG antibodies react specifically with anti-human-lgG conjugated to horseradish peroxidase (HRP). Within the incubation period of 30 min at RT, excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colorless substrate solution of 3, 3', 5, 5'-tetramethlyl-benzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution into the wells after 15 min at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound. The standard curve is established by plotting the antibodies concentrations of the calibrators (x-axis) and their corresponding OD values (y-axis) measured. The antibody concentration of the specimen is directly read off the standard curve. Alternatively, result can be calculated by a semi-quantitative method too using calibrator 2 as cut-off calibrator.

3.3.8.2.2Assay procedure

- Dilute patient sera with sample diluent (c)1+ 100(v/v), e.g. 10 µl serum + 1.0 ml sample diluent (c).
- Avoid any time shift during pipetting of reagents and samples.

1. Bring all reagents to room temperature (18-25 ˚C) before use. Mix gently without causing foam.

2. Dispense :100 µl calibrators 0 (optionally) 1-4 or 100 µl calibrator 2 (semi-quantitative), 100 µl control P,N (N optionally), 100 µl diluted patient samples into the respective wells.

3. Cover plate, incubate 60 min at room temperature (18-25 ˚C).

4. Decant, then wash each well three times using 300 µl wash solution (made of B).

5. Add 100 µl of conjugate (D) solution to each well.

6. Cover plate, incubate 30 min at room temperature (18-25 ˚C).

7. Decant, then wash each well three times using 300 µl wash solution (made of B).

8. Add 100 µl of substrate (E) to each well.

9. Incubate 15 min protected from light at room temperature (18-25 ˚C).

10. Add 100 µl of stop solution (F) to each well and mix gently.

11. Read the OD at 450 nm versus 690 nm within 30 min after adding the stop solution.

The construction of the standard curve for the measurement of anti-dsDNA in serum of SLE patients, for quantitative was carried by plotting the optical density of each calibrator with respect to the corresponding concentration values in IU/ml, figure (3.1).

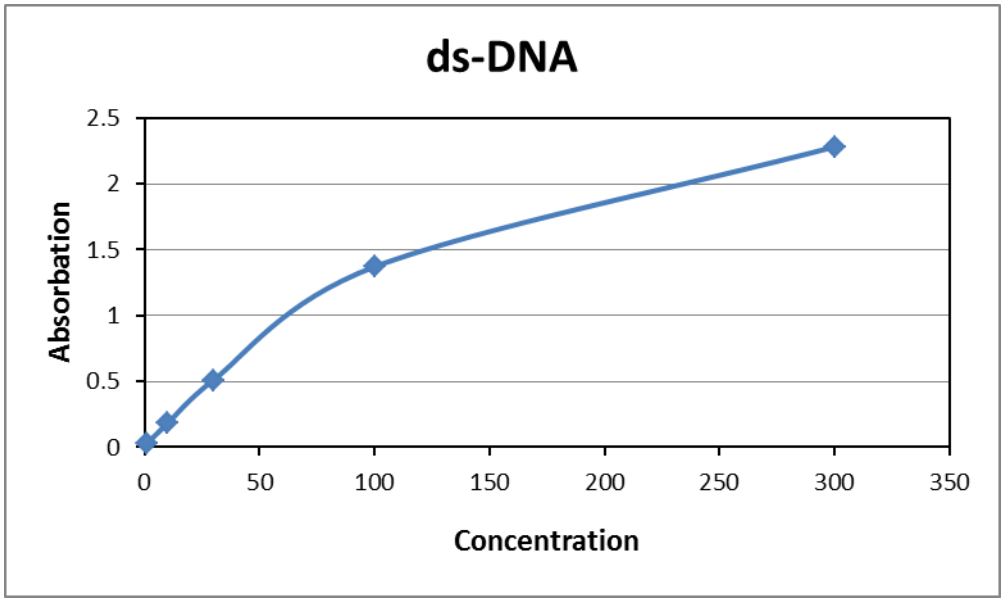


Figure 3.1: The stander curve of the anti-dsDNA.

3.3.9 Evaluation of Complements

Intended use for in vitro quantitative determination of complement component 3 and 4 (C3, C4) content in human serum or plasma of patients of SLE and control groups by radial Genius technique.

3.3.9.1 The principle of Complement test (C3, C4)

Complement factors (C3, C4) in the sample encounters its corresponding antibody in liquid phase, they immediately form an antigen-antibody complex with certain turbidity. With a certain amount of antibody, the turbidity is proportional to the antigen content in the sample. By detecting the reaction change at specific wavelengths and referring to the multi-point calibration curve, the complement (C3, C4) content in the sample can be calculated. The reagents are pre-calibrated, each specific calibration curve has been recorded into the magcard and each detection kit is allocated with one magcard.

3.3.9.2 Assay Procedure

1- After startup, the instrument displays the main measurement interface, select the test item and sample type at the time column

2- Click 'LOT ' at the batch No. column to enter the card-swiping interface. put the corresponding magcard onto the magnetic induction area, when a 'di ' sound heard , the magcard was successfully swiped, and the interface returns to the main measurement interface. For the same batch of reagents, it is no need to swipe the card again.

3- The instrument interface prompts ' Input Cupl '.

4- Take out cuvette, put one stirrer into it, and then use the pipettor to accurately add in 400 µl buffer solution, and then add in 3µl sample to C3 test and 4µl sample to C4 test.

5- Put the cuvette into the test channel, the instrument automatically stir for one time.

6- The instrument prompts ' please add antiserum ' then use the pipettor to accurately add (60 – 20) µl antiserum in (C3 –C4) Respectively.

7- Immediately press the corresponding channel's start button , the instrument will stir automatically. When the test finished, the instrument will automatically display and print the test result.

8- After the test, take out the cuvette; the instrument prompts ' input cup ', do the next test.

3.3.10 Evolution Human Thymosin β4

3.3.10.1 Principle of the assay

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for thymosin β4 has been pre-coated onto a microplate . Standards and samples are pipetted into the wells and any thymosin β4 present is bound by the immobilized antibody .After removing any unbound substances, a biotin-conjugated antibody specific for thymosin β4 is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of thymosin β4 bound in the initial step. Color development is stopped and the intensity of the color is measured.

3.3.10.2 Assay Procedure

1- Prepare all reagents, working standards, and samples as directed in the previous sections.

2- Refer to the assay layout sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the Ziploc, store unused wells at 4 ºC.

3- Add 100µl of standard and sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at 37 ºC. A plate layout is provided to record standards and sample assayed.

4- Remove the liquid of each well, don't wash.

5- Add 100µl of biotin-antibody (1x) to each well. Cover with a new adhesive strip. Incubate for 1 hour at 37 ºC (biotin-antibody (1x) may appear cloudy. Warm up to room temperature and mix gently until solution appears uniform).

6- Aspirate each well and wash, repeat the process two times for a total of three washes. Wash by filling each well with wash buffer (200µl) using a squirt bottle, multi-channel pipette , manifold dispenser, or autowasher, and let it stand for 2 minutes , complete removal of liquid at each step is essential to good performance. After the last wash ,remove any remaining wash buffer by aspirating ordecanting. Invert the plate and blot it against clean paper towels.

7- Add 100µl of HRP-avidin (1x) to each well. Cover the microtiter plate with a new adhesive strip. Incubate for 1 hour at 37 ºC.

8- Repeat the aspiration / wash process for five times as in step 6.

9- Add 90µl of TMB substrate to each well. Incubate for 15-30 minutes at 37 ºC Protect from light .

10- Add 50µl of stop solution to each well, gently tap the plate to ensure thorough mixing.

11- Determine the optical density of each well within 5 minutes using a micoplate reder set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. Subtract readings at 540 nm or 570 nm from the reading at 450 nm. This subtraction will correct for optical imperfections in the plate. Reading is made directly at 450 nm without correction may be higher and less accurate.

The construction of the standard curve for the measurement of thymosin β4 in serum of SLE patients, for quantitative was carried by plotting the optical density OD of each calibrator with respect to the corresponding concentration values in ng/ml, show in figure (3.2).

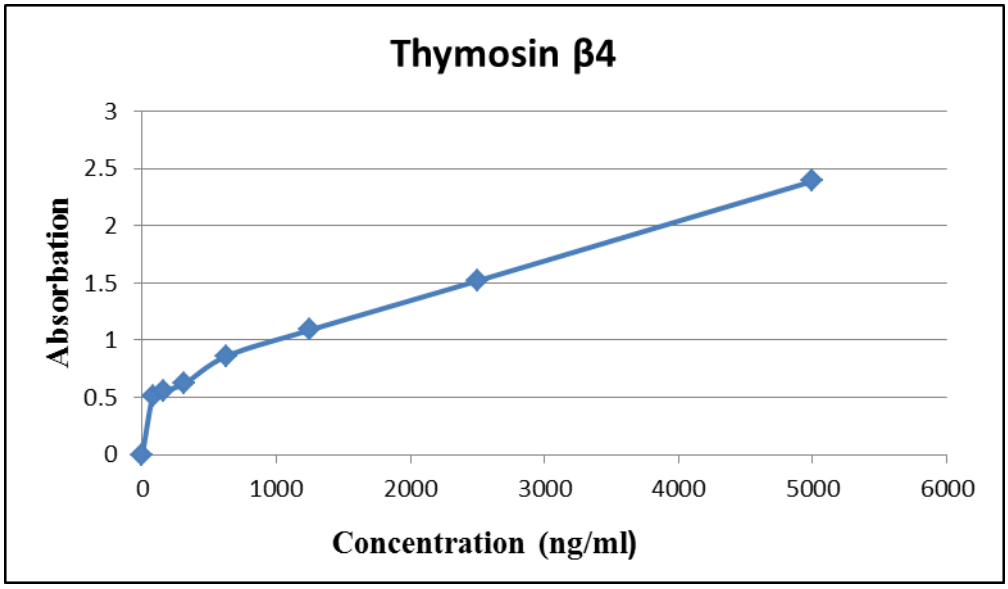


Figure 3.2: The stander curve of the thymosin β4 .

3.3.11 Determination of Quantitative 25-(OH)Vitamin D_3 by Enzyme-Linked Immunosorbant Assay (ELISA).

Vitamin D_3 was evaluated in serum of SLE patients and healthy control by using ELISA technique. Suspected vitamin D_3 deficiency or overdosing , reduced intestinal vitamin D_3 uptake, hypocalcaemia, hypophosphataemia, hypocalciuria, elevated alkaline phosphatase, reduced bone mineral content.

3.3.11.1 Principles of the test :

This ELISA test kit is designed for the in vitro determination of 25-OH vitamin D in human serum or plasma samples. In the first analysis step, the calibrators and patient samples are diluted with biotin-labeled 25-OH vitamin D and added to micro-plate wells coated with monoclonal anti-25-OH vitamin D antibodies.

During the incubation, an unknown amount of 25-OH vitamin D in the patient sample and a known amount of biotin-labelled 25-OH vitamin D compete for the antibody binding sites in the microplate wells plate. Unbound 25-OH vitamin D is removed by washing. For the detection of bound biotin-labelled 25-OH vitamin D, a second incubation is performed using peroxidase-labeled streptavidin. In a third incubation using the peroxidase substrate tetramethylbenzidine (TMB) the bound peroxidase promotes a color reaction. The color intensity is inversely proportional to the 25-OH vitamin D concentration in the sample. Result for the samples can be calculation directly using a standard curve.

3.3.11.2 Assay Procedure

- All reagents and specimens were transported to room temperature (25 ºC) before use.

- The calibrators/ controls and patient specimen were diluted 1:51 in working biotin.(Add 0.5 ml of working strength biotin to 10 µl of specimen and mix thoroughly by vortex).Incubate the mixture for at least 10 minutes to act at room temperature 25 ºC.

- Volume of 200 µl of sample diluted in biotin/ sample buffer was added into each of the microplate wells. Cover the microplate wells with the protective foil provided and incubated for 2 hours on microplate shaker 400 U/min at room temperature 25 ºC.

- The protective foils was removed and empty the wells, washed 3 times using 300 µl of working strength and buffer for each wash, the wash buffer was leaved in each well for 30 -60 second per washing cycle , then empty the wells. After washing, thoroughly disposed of all liquid from the microplate by tapping it on absorbent paper with the opening facing downwards to remove all residual wash buffers.
- The enzyme conjugate was pipette 100 µl into each of the microplate wells and incubated for 30 minutes on microplate shaker 400 U/min at room temperature 25 ºC.

- Empty the wells, washed as described above.

- Volume of 100 µl of chromogen/substrate solution was added into each of the microplate wells in the same speed as the chromogen/substrate solution was introduced.

- Volume of 100 µl of stop solution was added into each of microplate wells.

- Photometric measurement of the color intensity should be made at wave length of 450 nm within 30 minutes of adding stop solution. The results were recorded according to this table supplied by the Kit Company.

The stander curve from which the vitamin D_3 concentration in the serum sample can be taken is obtained by point to point plotting of the values measured for 6 calibrations sera. The optical density of each calibrator with respect to the corresponding concentration values in ng/ml, show in figure (3.3).

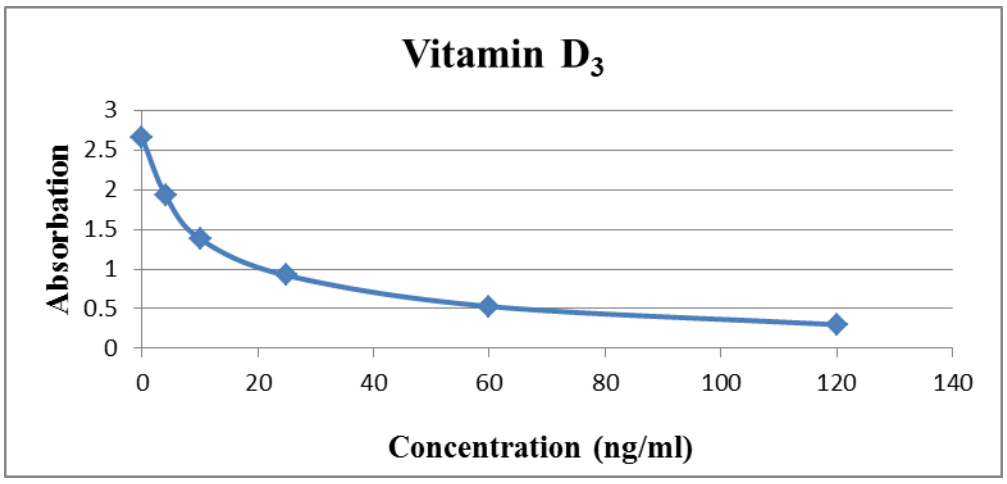


Figure 3.3 : The stander curve of the Vitamin D_3.

3.3.12 Cystatin C :

Intended used : ST AIA-PACK Cys-C is designed for in vitro diagnostic use only for the quantitative measurement of Cys-C in human serum, heparinized plasma or EDTA plasma on TOSOH AIA System Analyzers. Cys-C measurement is used as an aid in the diagnosis and treatment of renal disease.

3.3.12.1 Principle test

The ST ALA-PACK Cys-C is a two-site quantitative fluorescent immunoassay which is performed entirely in the ST ALA-PACK Cys-C test cups. Cys-C present in the test sample is bound with monoclonal antibody immobilized on magnetic beads and enzyme-labeled monoclonal antibody. The magnetic beads are washed to remove unbound enzyme-labeled monoclonal antibody and are then incubated with fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled monoclonal antibody that binds to the beade is directly proportional to the Cys-C concentration in the test sample. A standard curve is constructed, and unknown sample concentration are calculated using this curve.

3.3.12.2 Assay procedure

1. **Reagent preparation**

a. Substrate solution

Bring all reagents to 18-25˚C before preparing the working reagent. Add the entire contents of the AIA-PACK SUBSTRATE RECONSTITUENT II (100ml) to the lyophilized AIA-PACK SUBSTRATE RECONSTITUENT II and mix thoroughly to dissolve the solid material.

b. Wash solution

Add the entire contents of the AIA-PACK WASH CONCENTRATION (100ml)to approximately 2.0L of CAP class I water or the clinical laboratory reagent water (formerly NCCLS type I) defined by CLSI C3-A4 guideline, mix well, and adjust the final volume to 2.5L.

c. Diluent

Add the entire contents of the AIA-PACK DILUTION CONCENTRATION (100ml)to approximately 2.0L of CAP class I water or the clinical laboratory reagent water (formerly NCCLS type I) defined by CLSI C3-A4 guideline, mix well, and adjust the final volume to 5.0L.

2. Calibration procedure

a. Refer to the appropriate TOSOH AIA system operator's manual for the procedural instructions.

b. Verify that both the calibrator lot and concentration numbers have been correctly entered into the software.

c. The AIA-PACK Cys-C CALIBRATOR SET is lyophilized. All levels should be reconstituted with 1.0 ml of CAP Class I water or

the clinical laboratory reagent water (formerly NCCLS type I) defined by CLSI C3-A4 guideline.

d. TOSOH recommends that all calibrators be run in triplicate.

3. Specimen processing

a. Preparation

Following the specific instructions in the operator's manual for the analyzer, place samples on the instrument appropriately.

b. Assay procedure

1. Ensure a sufficient quantity of ST AIA-PACK Cys-C test cups for the number of samples to be run.

2. Load patient samples as instructed in the operator's manual and proceed with analysis.

The construction of the standard curve for the measurement of cystatin C in serum of SLE patients, for quantitative was carried by plotting the optical density OD of each calibrator with respect to the corresponding concentration values in mg/L , as figure (3.4).

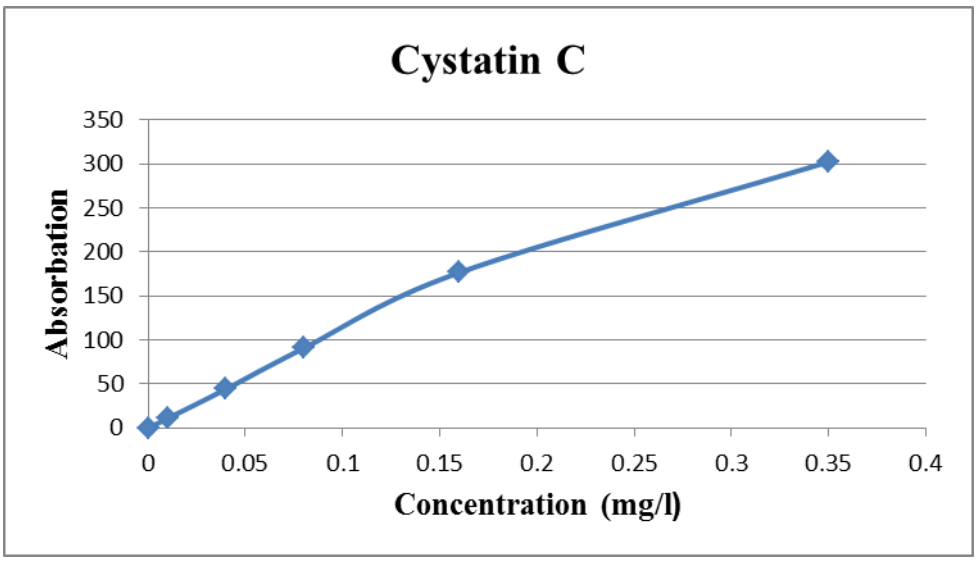


Figure 3.4: The stander curve of Cystatin C.

3.3.13 The Biochemical test:

3.3.13.1 Urea test

Intended use: For the quantitative in vitro determination of Urea in serum, plasma and urine. This product is suitable for manual use.

3.3.13.1.1 Colorimetric method

- **Principle**

  The method is based on the following reaction:

  $$Urea + H2O \longrightarrow 2NH3 + CO2$$

  Salicylate and hypochlorite in the reagent react with the ammonium ions to form a green complex.

- **Reagent**

Contents		Initial concentration

RI a.	urease	≥ 5000 U/l
RI b.	phosphate buffer	120 mmol/l , pH 7.0
	Sodium salicylate	63.4 mmol/l
	Sodium nitroprusside	5.00mmol/l
	EDTA	1.5 mmol/l
R2.	Sodium hypochlorite	18 mmol/l
	Sodium hydroxide	750 mmol/l
CAL	Standard	See lot specific insert

- **Stability and preparation of reagents**

Urease Rla , phosphate buffer Rlb , sodium hypochlorite R2 and standard (CAL) are supplied ready to use. Stable up to the expiry date stated when stored at +2 to +8 ˚C .

- **Working reagent**

Add 1 vial of urease Rla to 1 bottle of phosphate buffer Rlb. Stable for 1 month at +2 to +8 ˚C stored protected from light.

- **Procedure**

Wavelength	600nm (Hg 578 nm – Hg 623 nm)
Cuvette	1 cm light path
Temperature	+ 25 , + 37 ˚C

Measurement	Against reagent blank

Pipette into test tubes :

	Reagent blank	Standard	Sample
Standard	--------	10µl	-------
Sample	--------	------	10µl
Working reagent (Rl)	1 ml	1 ml	1 ml

Shake. Incubate for at least 3min at 37˚C or 5 min at 20 to 25 ˚C.

- **Calculation**

Urea concentration= Asample /A standard * standard conc. (mmol/l)

(mmol/l)

Urea concentration= A sample/A standard* standard conc.(mg/dl)

(mg/dl)

- **Normal values**

Serum or plasma	2.5 – 7.5 mmol/l (0.15 – 0.45 g/l)
Urine	338 – 538 mmol/24h (20 -35 g/24h)

3.3.13.2 Uric acid:

Intended use: For the quantitative in vitro determination of uric acid in serum, plasma, urine. This product is suitable for manual use and on the Rx Monza analyser .

3.3.13.2.1 Colorimetric method

Uric acid is converted by uricase to allantoin and hydrogen peroxide, which under the catalytic influence of peroxidase, oxidizes 3,5-dichloro-2-hydroxybenzenesulfonic acid and 4-aminophenazone to form a red-violet quinoneimine compound.

- **Principle**

  Uric acid + O_2 + $2H_2O$ ⟶ Allantoin + CO_2 + H_2O

  $2H_2O$ + 3,5-dichloro-2-hydroxybenzenesulfonic acid + 4-aminophenazone ⟶ N-(4-antipyryl)-3-choro-5-sulfonte-p-benzo-quinoneimine

- **Reagent composition**

Contents	**Concentration in the test**
Rla. Buffer	
Hepes buffer	50 mmol/l , pH 7.0
3,5-dichloro-2-hydroxy-benzenesulfonic acid	4 mmol/l
Rlb. Enzyme reagent	
4-Aminophenazone	0.25 mmol/l
Peroxidase	≥ 1000 U/l
Uricase	≥ 200 U/l
CAL. Standard	See lot specific insert

- **Stability and preparation of reagent**

  Rla. Buffer : contents ready for use. Stable up to the expiry date when stored at +2 to +8˚C

Rlb. Enzyme reagent : UA 230 reconstitute one vial of enzyme reagent Rlb with 15 ml of buffer Rla. Stable for 21 days at +2 to +8 ˚C or 5 days at +15 to +25 ˚C stored protected from light.

CAL. Standard : contents ready for use. Stable up to expiry date when stored at +2 to +8 ˚C .

- **Procedure**

Using fresh ddH2O to perform a new gain calibration in cuvette mode. Select UA in the run test screen and carry out a water blank as instructed.

Pipette into a cuvette:

	Reagent blank SO	Standard S1	Sample
ddH2O	--------	-------	------
Standard	---------	10 µl	------
Sample	---------	-------	10 µl
Reagent	500 µl	500 µl	500 µl

Mix, incubate for 15 min at 20-25 ˚C or 5 min at 37 ˚C.

Insert into the RX monza flowcell holder and press read within 30 mins.

- **For manual use**

Wavelength	520 nm ;Hg 546 nm
Cuvette	1 cm light path
Reaction temperature	20-25 ˚C /37˚C
Measurement	Against reagent blank only one reagent blank per series is required

Pipette in to test tube

	Reagent blank (µl)	Sample (µl)	Standard (µl)
Sample	--------	20	------
Standard	--------	-------	20
Reagent	1000	1000	1000

Mix , incubate for 15 min at 20 -25 ˚C , or for 5 min at 37 ˚C.

Measure absorbance of sample and standard against reagent blank within 30 min.

- **Manual calculation**

  Serum or plasma

  Uric acid concentration = standard conc.× A sample /A standard (mg/dl)

- **Normal values**

  Serum : men 3.4 – 7.0 mg/dl

  Women 2.4 – 5-7 mg/dl

3.3.13.3 Creatinine

Creatinine forms in alkaline solution an orange-red coloured complex with picric acid. The absorbance of this complex is proportional to the creatinine concentration in the sample.

- **Principle**

  Creatinine + picric acid ⟶ creatinine – picric complex

- **Assay procedure**

  Wavelength : Hg492 nm (490 – 510 nm)

  Optical path : 1 cm

  Temperature : 25 ˚C / 37 ˚C

  Measurement : against air (increasing absorbance)

Sample / STD : 100 µl

Working reagent : 1000 µl

Mix and start the stopwatch. After 30 sec. read the absorbance A1. Read the absorbance A2 exactly after 2 min.

3.3.14 Statistical Analysis

Statistical analysis was performed using statistical package for the social sciences (SPSS) statistical software for windows. The results are presented as means and standard deviation (SD). Comparison of group differences on normally distributed numerical variables were assessed by using the students' T-test to compare between patients and healthy control (group1 and 2) and ANOVA, one way to compare between classes of patients (group 1,2) depended on the least significant difference (LDS) at level less than 0.05. P-values at levels ($p<0.05$) was considered to be statically significant. Correlation between parameter were determined using the Pearson correlation coefficients.

4. Results and Discussions

4.1 Sample distribution

4.1.1 gender incidence

The majority of SLE patients were 57 females and 3 males, the ratio of female to male 19:1. In the healthy control 27 females and 3 males, the ratio of female to male 9:1. Shown in Figure (4.1).

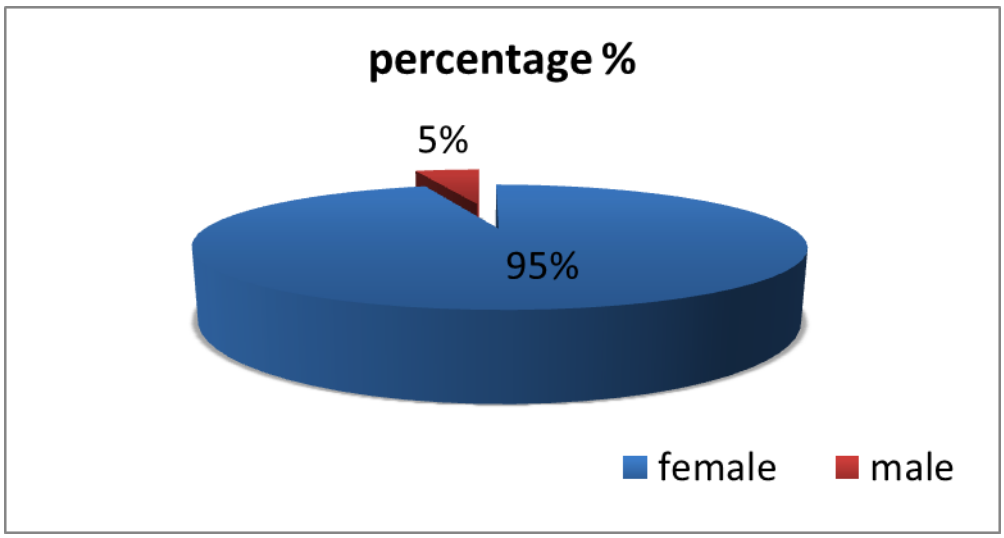


Figure 4. 1: Distribution of SLE patients according to gender .

It has been found that female of all mammalian species have an increased incidence of autoimmune inflammatory disease compared to males, and the presence of estrogen increases the severity of autoimmune disease. (Isgor *et al.*, 2003).

This result in agreement with most studies in the world showed wide range of female: male (Salido, 2010 and Pradgan *et al.*, 2013).

The increased female to male ratio of SLE patients suggests that sex factors modulate disease proclivity and development (Whitacre *et al.*, 1999), this disease related with steroid hormones, especially estrogen and progesterone, influence the immune system. Low progesterone levels in women with lupus, suggesting that a relative imbalance in favor of estrogen may contribute to immune reactivity in some female patients (Shabanova, 2008).

4.1.2 Age group

The age of patient with SLE in this study (n= 60) ranged between (7-65) years with the mean age (**36.11** ±1.53) compared with healthy control (n=30) ranged (7-65) years with the mean age (**37.56** ±2.32) .

The age group of SLE patient from (<15) years reported 1 (1.7%) while the age group (15-45) years reported 47 (78.3%) and 12 (20%) belong to (>45) years age group, as shown in figure (4.2).

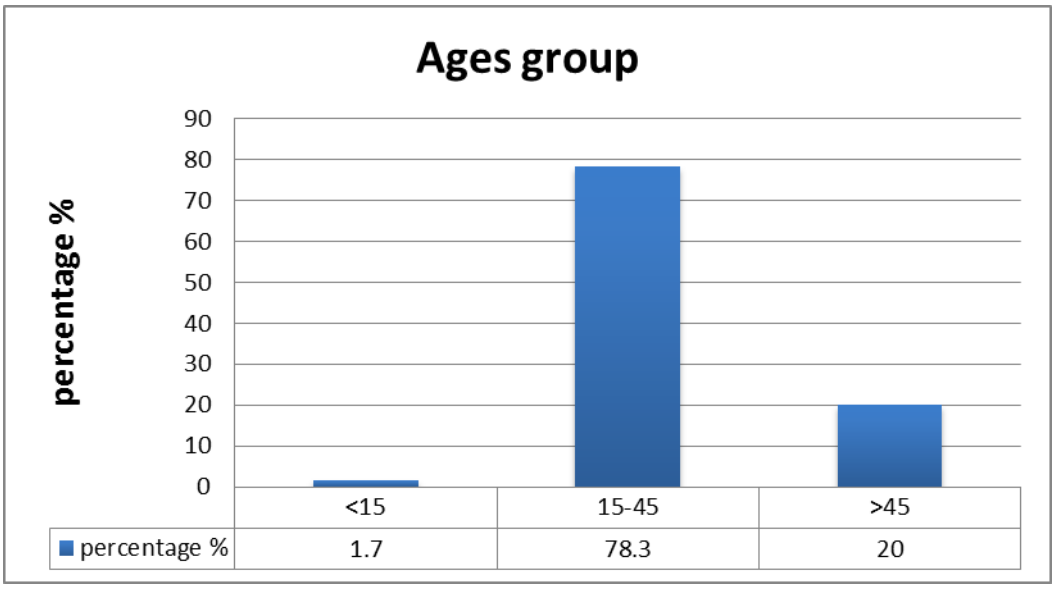


Figure 4. 2: Distribution of SLE patients according to age group.

The current study showed that the demographic distribution of data showed that SLE was teenager and old age women less than women in fertilizing period (> 15 & < 45) year, may contribute to the psychiatric disorder and anxiety . This result agreement with the results published by many studies in Iraq and other countries (Kllippel, 1997; Hassan, 2000).

The age group (15-45) years whether females or males was more affected statistically than other age group. This result according with studies in Southern Asia And other studies in Eastern Asia shows that, SLE disease can affect all ages but most commonly the age group (15-45) years (Heller *et al.*, 2007; AlSaleh *et al.*, 2008; Wang *et al.*, 2007; Mok *et al.*, 2008 and Salido and Manapat-Reyes, 2010).

4.1.3 Clinical manifestation

According to the American College of Rheumatology (ACR) clinical criteria of SLE patients, arthritis was the most frequent feature , which was

observed in (95%) of patients, hematological disorder (90%), oral ulcer (80%), immunological disorder (75%), photosensitivity (60%), molar rash (50%), kidney disorder (21.6%), serositis (15%) and Neurologic disorder (10%). As show in Figure (4.3).

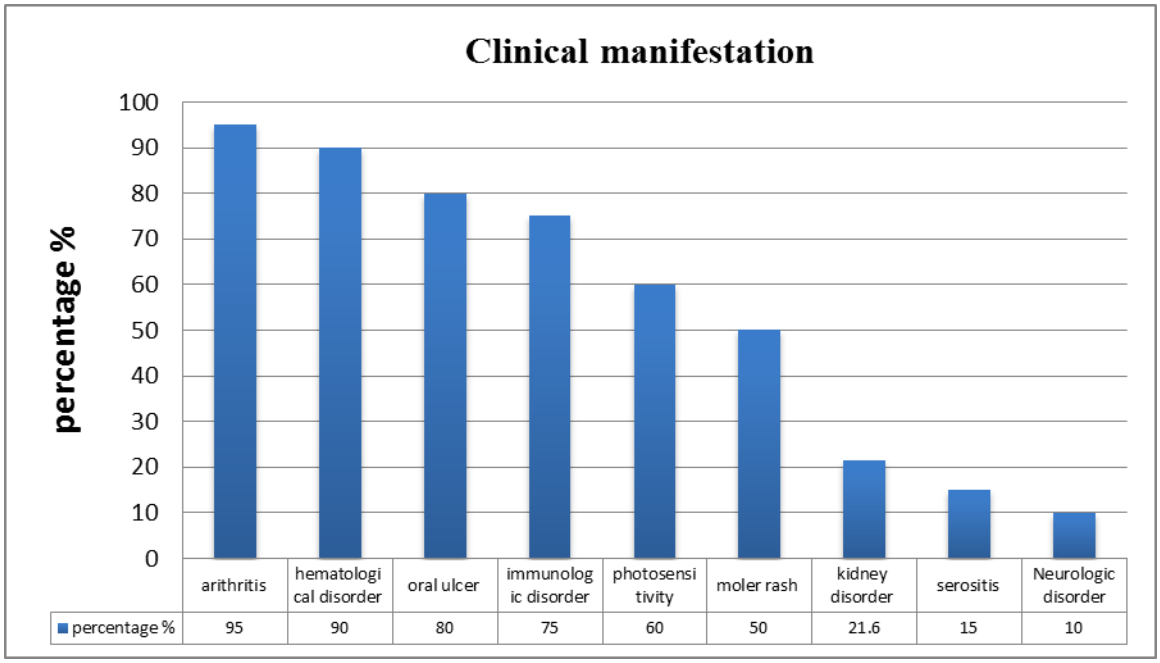


Figure 4. 3:The clinical manifestation of SLE patients during their disease duration.

This study has shown that prevalence of some manifestation such as arthritis, malar rash, oral ulcer and kidney disorder were high than other criteria. Arthritis is the most common ACR-defined clinical criterion for SLE diagnosis, this finding was similar to some studies in Arabic countries (Heller *et al.*, 2007; AlSaleh *et al.*, 2008), also recent studies in Eastern Asia (Wang *et al.*, 2007 and Salido and Manapat-Reyes, 2010).

In addition of this criteria several symptoms were common in Iraq population, including fever, joint pain, fatigue and weight loss. This finding matches with some recent studies (Pradgan *et al.*, 2013).

4.1.4 SLE Disease Activity Index (SLEDAI)

According to disease activity (SLEDAI), SLE patients can be divided into three groups, mild, progressive and lupus nephritis. The current data

showed that 33 (55%) of patients with mild state ranged from (16-65), 14 (23.3%) with progressive state which ranged from (19-58) and 13 (21.6%) with lupus nephritis state ranged from (7-55). As shown in Figure (4.4).

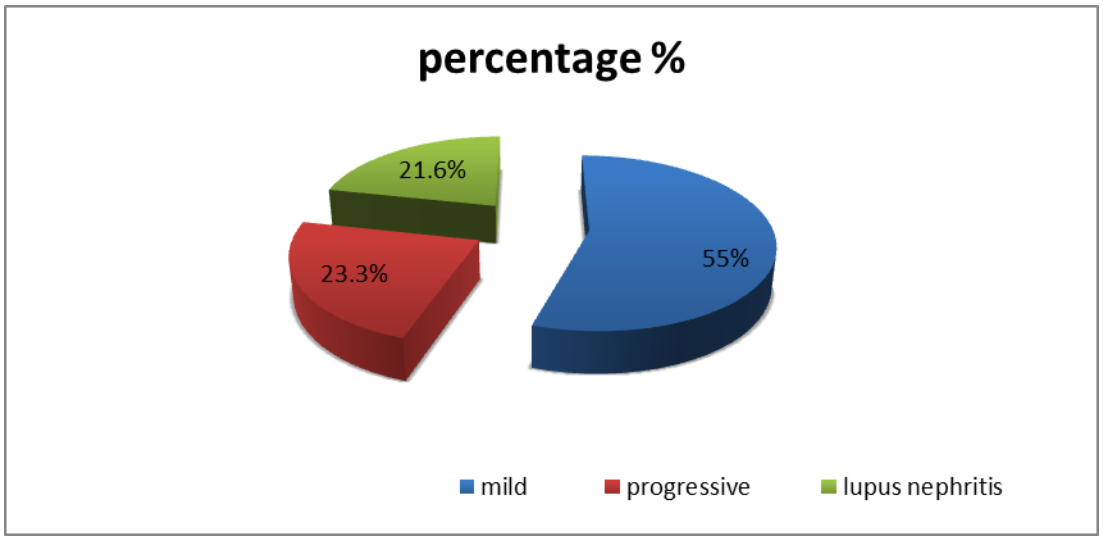


Figure 4. 4 : The distribution of SLE patients depended on SLEDAI.

Criteria of SLEDAI depended mainly on the primary symptom presentation that varied widely from patient to patient and the essential diagnoses test to recognize active and inactive of SLE disease. (Urowitz and Gladman, 1998 and Narayanan *et al.*, 2010) and the third group is a sub division from active group lupus nephritis .

Examining patients for potential autoimmune disease such as SLE disease is fraught with difficulty because not only a one laboratory test can the detection establish. Typically, but there must be a multiple laboratory tests are needed including a basic studies like immunological test, biochemical test and hematological test.

4.2.1 Seroimmunological data

4.2.1.1 Serum level of the selected autoantibodies

4.2.1.1.1 Anti- Nuclear Antibodies (ANA)

The positive ANA was observed in 42 (70%) when the index value > 1.1, while negative ANA present in 18 (30%) when index value < 0.97 as shown in figure (4.5).

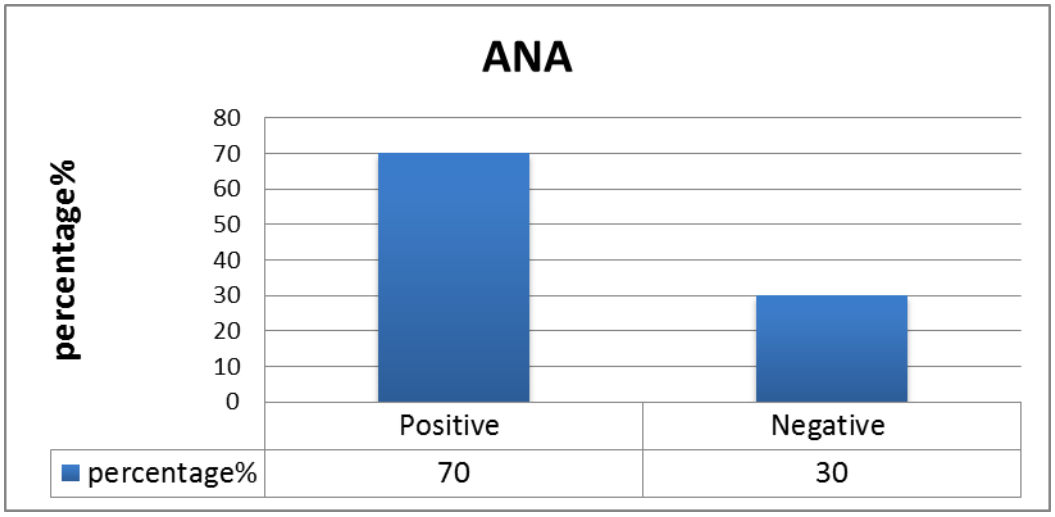


Figure 4. 5: ANA Ab detection in serum of SLE patients .

The mean of ANA for SLE patients (**2.65** ±0.33) was statistically highly significant at $p < 0.05$ when compared to the mean of healthy controls (**0.3** ±0.02), also observed that ANA concentration is higher among the serum of lupus nephritis (**6.73** ±0.67 IU/ml) in comparison with mild and progressive (**1.02** ±0.09 ; **2.70** ±0.12 IU/ml**)** respectively $P \leq 0.05$**,** figure (4.6).

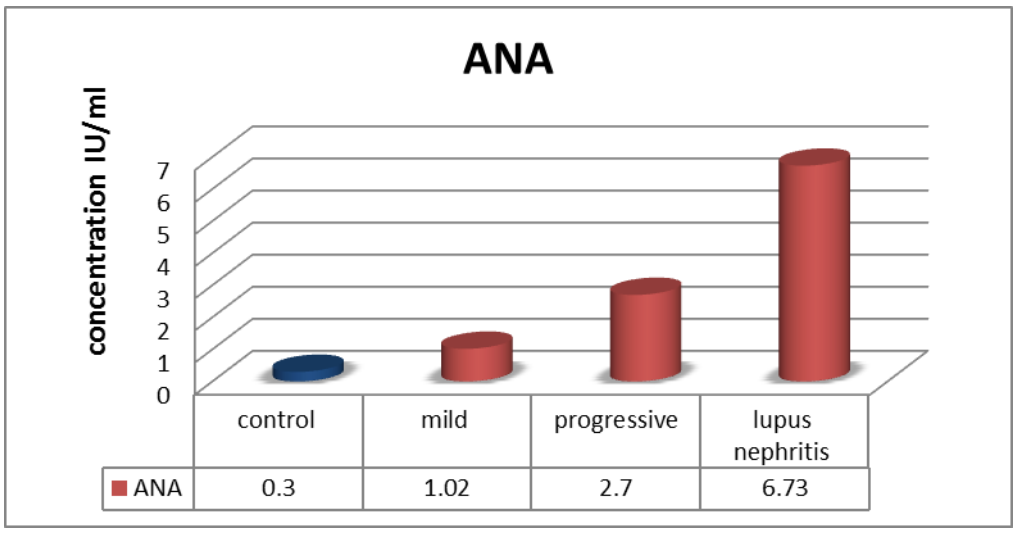


Figure 4. 6: The concentration of ANA in serum of SLE patients and healthy control.

This study demonstrated that high titers of ANA are most often associated with active SLE.(Kavanaugh, 2000) said that this test is one of the most common tests used by physicians to diagnosis SLE. The anti-nuclear antibody (ANA) are heterogeneous group of antibodies produced against variety of antigens within the cell nucleus .

A positive ANA test does not automatically mean lupus but it shows that immune system is making an antibody that reacts with components of body's cells, ANA positive is not mandatory for the diagnosis, because most people with SLE have ANA, but most patients with ANA do not have SLE. and may not relate to the patients symptoms but were indicated to other autoantibodies that may present in patient serum. (Kavanaugh *et al.*, 2000 and Hyoun *et al.*, 2009).This finding was similar to many studies (William, 2000 ; Aysun *et al.*, 2010 and Maher, 2013).

4.2.1.1.2 Anti-double strand deoxyribonucleic acid (anti-dsDNA)

The curve of anti-dsDNA may explain that each patient has his own concentration that differs from other. Positive anti-dsDNA was found in 53 (88.3%), patients with 7 (11.7%) negative anti-dsDNA as shown in figure (4.7).

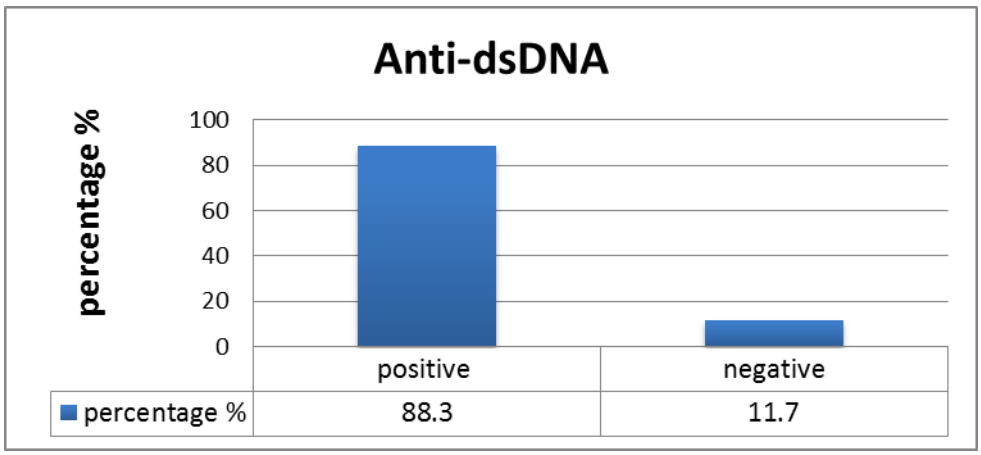


Figure 4. 7: Detection of anti-dsDNA in serum of SLE patients.

The results of current study have illustrated a significant difference in the serum level of anti dsDNA in patients with SLE disease and healthy group. It is clear that anti dsDNA has been raised in the serum of SLE patients (**222.29** ±31.42 IU/ml) ranged (12.5 - 1022 IU/ml) as comparison with healthy control (**10.2**7 ±0.97 IU/ml) ranged (2.4 -24.1 IU/ml), also observed that dsDNA concentration is higher among the serum of lupus nephritis (**503.9** ±85.98 IU/ml) in comparison with mild and progressive (**84.35** ±27.25 ; **285.9** ±33.14 IU/ml**)** respectively $p \leq 0.005$ as shown in figure (4.8).

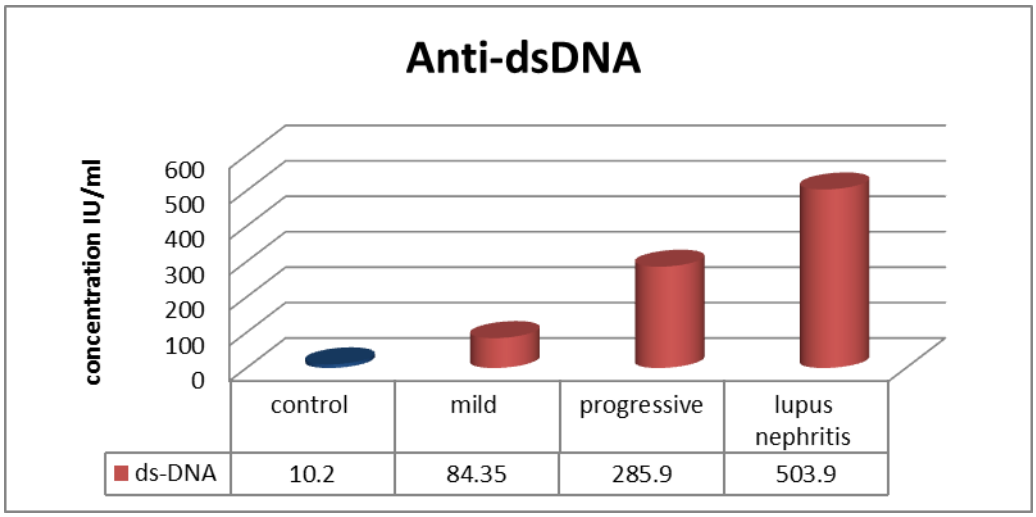


Figure 4. 8: Concentration of ds-DNA in SLE patients and healthy control.

Antibodies to dsDNA in serum of patients are elevated compared with the control, these results show that increase in anti dsDNA antibody

concentration prior to disease exacerbations of SLE are part of a restricted immune response or merely the consequence of polyclonal B cell activation(Ter Borg *et al*., 1991) . Moreover Giasuddin *et al*., (1991) have mentioned that the anti dsDNA antibodies are present in 85.3% of SLE patients.

The study displayed that high titers of anti-dsDNA are seen primarily in serum of SLE patient and closely parallel with the disease activity especially in lupus nephritis than other group, and this finding was in agreement with many studies (Edwards, 2001; Mok and Lau, 2003 and Rabbani, 2006).

For many years, the anti-dsDNA antibody assay has been regarded as the serological gold standard in the diagnosis and assessment of disease activity in patient with SLE (Ho *et al.*,2001 ; Isenberg , 2004). The prevalence of anti-dsDNA in this study was 75%. This is in consistent with previous data which reported that anti-dsDNA reactivity was between 40 -80% of SLE patients (Ravirajan *et al.,* 2001).

Increased levels of anti-dsDNA antibodies are related with disease flares usually in combination with decreased levels of the complement proteins C3 and C4(Linnik *et al*.,2005 ; Ng *et al*.,2006).

4.2.1.2 The Complements (C3, C4)

The result of C3 and C4 concentration in SLE patient's sera showed significant reduction comparing to healthy control. The mean concentration of C3 for SLE patients were (**1.47** ±0.1 g/l) ranged (0.26 – 3.13) while the mean concentration of C4 for SLE patients was (**0.2** ±0.03 g/l) ranged (0.01-0.63) in compared to the mean concentration of healthy controls were (**1.65** ±0.11 ; **0.36** ±0.04g/l) ranged (0.40 – 2.64 ; 0.12 – 1.40) respectively, as shown in figure (4.9)(4.10).

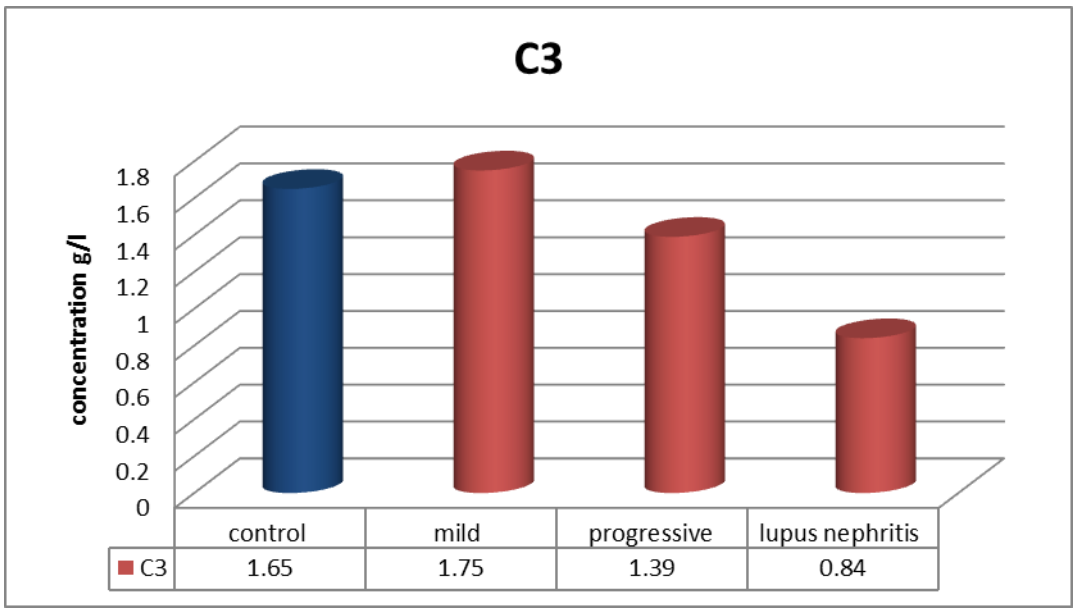


Figure 4. 9: Concentration of complement system (C3) in SLE patients and healthy control .

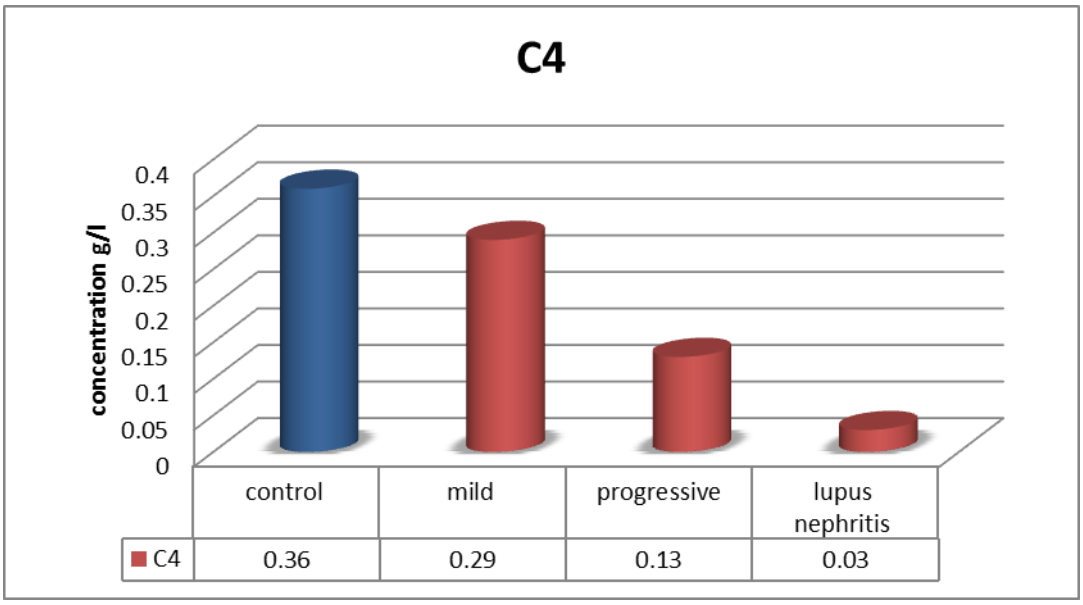


Figure 4. 7: Concentration of complement system (C4) in SLE patients and healthy control .

This result shows that the level of complement (C3 and C4) in SLE patients was significantly decreased compared to the healthy control , and C4 level more related with lupus nephritis. This result is in agreement with other reported studies (Hassan, 2000 ; Abd, 2010).

These results are compatible with results of (Li *et al*., 2015), who have found low serum level of complement C3 and C4 in Chinese patients with SLE.

Also Julkunen *et al*., (2012) suggests that complementC3 and C4 serum levels are correlate with disease activity and patients with active lupus nephritis had significantly reduced levels of C3 and C4 compared to patients with inactive lupus nephritis.

The present study found a relationship between C3 or C4 serum levels and renal flares. In a particularly C4 is critical for starting a renal flare, while C3 activation is implicated in the actual tissue damage, (Birmingham *et al*., 2010)**.**

In addition Ho *et al*. (2001) have found that decreases in serum levels of complement were not consistently related with SLE flares but also related with a concurrent increase in renal and hematologic SLE activity.

4.3 Hematological Criteria

4.3.1 Erythrocytes Sedimentation Rate

This study showed that SLE disease was associated with raised ESR and the mean was (**64.1** ±3.81 mm/h) that range (20 -120 mm/h) in SLE patients when compared to healthy controls (**13.8** ±1.1 mm/h) range (5-30 mm/h), also observed that ESR concentration is higher among the serum of lupus nephritis (**86.18** ±6.85 IU/ml) in comparison with mild and progressive (**50.93** ±4.6 ; **74.64** ±8.18 IU/ml**)** respectively the difference was statically significant $p \leq 0.05$, figure (4.11).

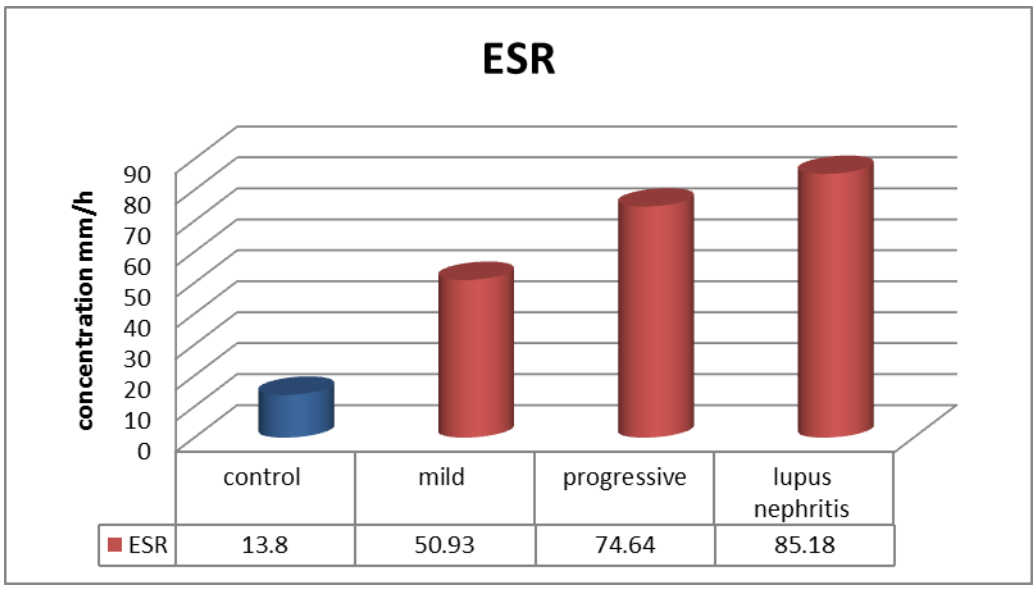


Figure 4. 8: Level of ESR in SLE patients and healthy control.

The result showed increasing level of ESR in SLE patients compared with healthy control, this elevation due to inflammation causes an increase in the ESR, and this result match with (Shaldonum *et al.*, 2012; Afify *et al.*, 2013 and Kusworini *et al.*, 2013).

Multiple factors influence the ESR include patient's age, sex, RBC morphology, hemoglobin concentration, in spite of ESR is not a specific test, but it can be used to monitor disease activity and treatment response and signal that inflammatory or infectious stress is present (Lahita and Weinstein, 2007 ; Klippel *et al.*, 2008).

ESR test measured the quantity of red blood cells (RBC) which precipitate in a tube at a defined time that is based upon serum protein concentration; and when RBC interact with these proteins the level will rise (Kapoor, 2010).

4.4 Parameters related with SLE

4.4.1 Evolution thymosin β4 in SLE

The mean concentration of thymosin β4 for SLE patients was (**177.15** ±50.1) compared to average of healthy control was (**575.4** ±79.58), also observed that thymosin β4 concentration is decrease among the serum of lupus nephritis (**42.41** ±36.2 IU/ml) in comparison with mild and progressive (**245.42** ±69.86 ; **141.33** ±124.7 IU/ml**)** respectively the difference was statically significant $p \leq 0.05$ as shown in figure (4.12).

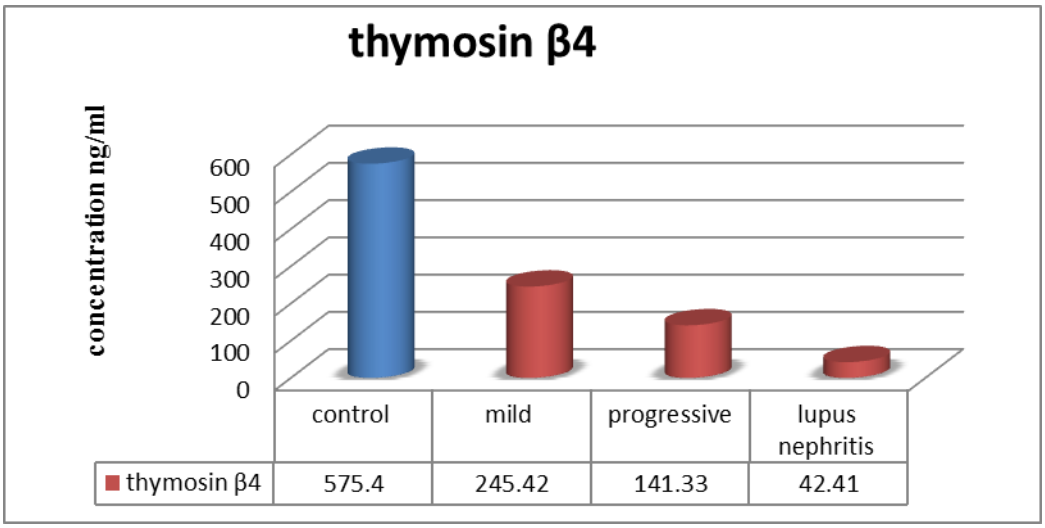


Figure 4. 9: Concentration of thymosin β4 in SLE patients and healthy control .

This result showed that the level of thymosin β4 was significantly decreased in SLE patients compared with healthy control, and more related with lupus nephritis. Other study suggested that thymosin β4 decreased with flare-up, this result agreed to other study (Hussain *et al.*, 2008)**.**

The result showed positive correlation which show a significant decreasing in concentration of thymosin-β 4 and circulating complement factors (C3&C4) respectively in active SEL compare in control respectively and positive correlation which show a significant increasing in ESR. (Hussain *et al.*, 2008)

4.4.2 Evaluated of vitamin D3 levels .

The mean of vitamin D_3 in the SLE patients was (**13.52** ±1.3 ng/ml) ranged from (5.5 – 32.9 ng/ml) compared with healthy control (**24.80** ±1.26 ng/ml) ranged (13.00 -35.00 ng/ml).), also observed that vitamin D_3 concentration is decrease among the serum of lupus nephritis (**8.95** ±2.12 IU/ml) in comparison with mild and progressive (**16.34** ±1.1 ; **11.12** ±4.19 IU/ml**)** respectively the difference was statically significant $p \leq 0.05$, as shown in figure (4.13).

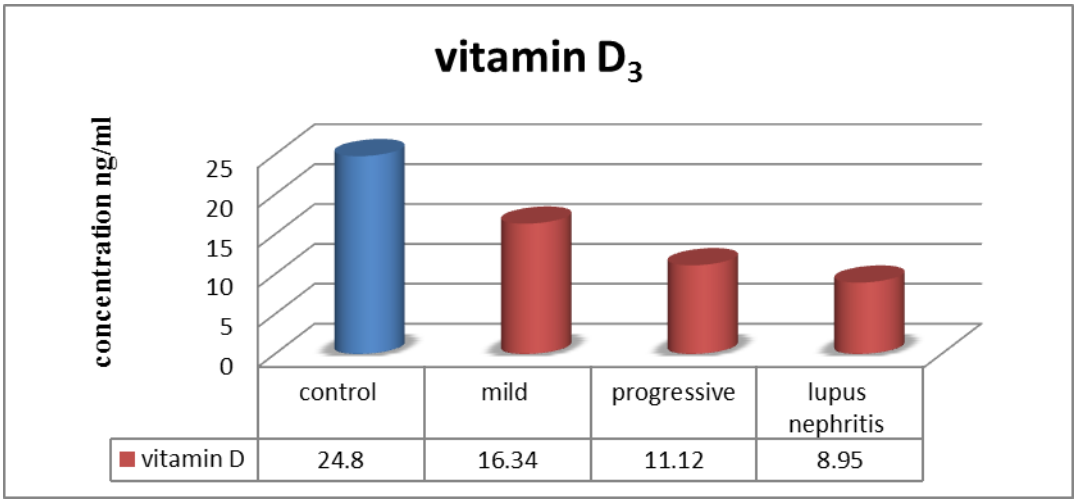


Figure 4. 10: Concentration of Vitamin D_3 in SLE patients and healthy control.

The result exhibited that low vitamin D_3 concentration was frequent in SLE patients, and indicated that SLE patients had higher risk of insufficient vitamin D_3. The high prevalence of SLE patients who have vitamin D_3 concentration below normal was similar with most studies in the world (Kim *et al.*, 2010 ; Mok *et al.*, 2012 and Robinson *et al.*, 2012).

The low level of vitamin D_3 causes impaired immunological response that is thought to increase disease activity in SLE (Cutolo and Otsa, 2008). Vitamin D_3 deficiency is worldwide problem with serious health effects such as SLE disease and one of the most important risk factors for immune system. (Lips, 2006).

Furthermore, low vitamin D_3 level was found as strong predictor of cutaneous lupus (Cutillas- Marco *et al.*, 2010).

Table 4. 1: Comparison between two groups of SLE patients before and after receiving Ca+ / vitamin D_3 depend on serum level of(ANA , ds-DNA, C3,C4) and ESR with serum level of 25-OH D3 .

	ANA	ds-DNA	ESR	C3	C4	Vitamin D_3
Group1	**SLE patients before receiving Ca+/ Vitamin D_3**					
Mean ±SE	**6.73** ±0.67	**503.9** ±85.98	**86.18** ±6.85	**0.84** ±0.17	**0.03** ±0.02	**8.95** ±2.12
Rang	4.10- 10.60	253.8- 1022.50	55.00– 110.00	0.26- 1.74	0.01- 0.1	5.50 – 17.30
Group2	**SLE patients after receiving Ca+/ Vitamin D_3**					
Mean ±SE	**3.35** ±1.44	**228.23** ±92.70	**43.23** ±12.81	**1.14** ±0.44	**0.12** ±0.09	**17.77** ±4.93
Rang	1.80- 6.00	60.00-389.00	20.00-67.00	0.68- 1.90	0.04- 0.40	13.00-28.00

This result shows significantly decrease in criteria of SLE patients compared with healthy control, and more related with lupus nephritis (Robinson *et al.*, 2012). After treated with Ca+/ Vitamin D_3 the result show an increasing in the concentration of vitamin D_3 and this increasing depend on level dose, but stay the level of vitamin D_3 less than normal value.(Al-Ahmed, 2014).

4.5 Biochemical Test :

4.5.1 Evaluation Cystatin C in SLE

The mean concentration of cystatin C for SLE patients was (**2.32** ±0.11) range (0.89 – 4.80) compared to average of healthy control was (**0.60** ±0.03) range (0.39 – 1.35), also observed that Cys.C concentration is increase among the serum of lupus nephritis (**2.83** ±0.33 IU/ml) in comparison with mild and progressive (**2.11** ±0.12 ; **2.34** ±0.18 IU/ml**)** respectively the difference was statically significant $p \leq 0.05$, as shown in figure (4.14).

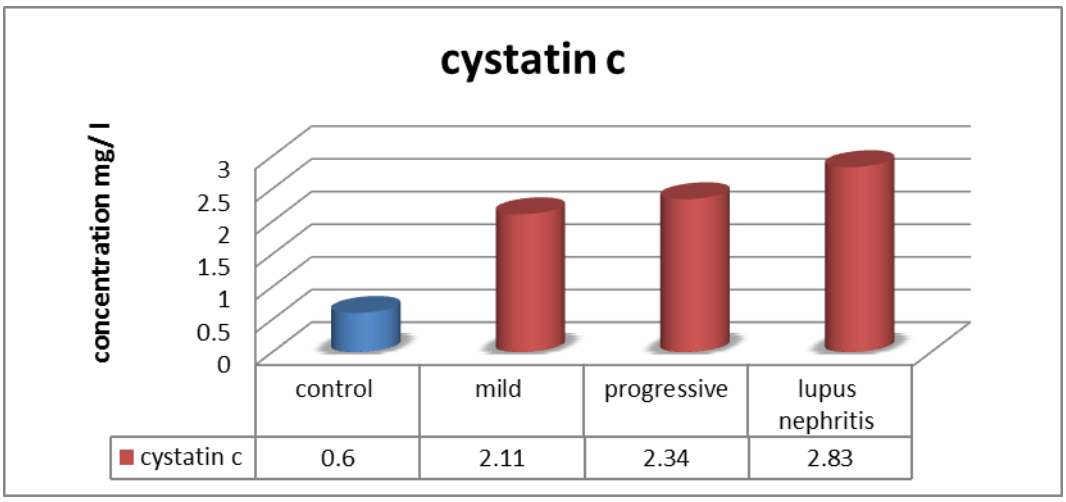


Figure 4. 11:Concentration of Cystatin C in SLE patients and healthy control .

This result shows that concentrations of Cys-C were significantly higher in patients with SLE than in healthy control. Furthermore, Cys-C, but not other measures of renal function, was significantly correlated with inflammatory markers and disease activity in SLE, also Cys-C is not affected by gender and muscle mass because of its production rate is usually constant, its plasma concentration reflects GFR (Shlipak *et al.*, 2005).

4.5.2 Urea, Uric Acid and Creatinine

Biochemical investigations were done for measure the chemical substances carried by the blood. Urea ,uric acid and creatinine commonly used to assess kidney function as renal injury (glomerulonephritis). In the current study the mean of urea in the SLE patients was (**45.18** ±3.69 mg/dl) ranged from (18.50- 155.00mg/dl) compared with healthy control (**31.07** ±1.1 mg/dl) ranged (21.00 - 43.00mg/dl). There was significant difference between the urea in SLE patients and healthy control $p< 0.05$, as shown in table (4.15).

Also, the study found high concentration of both uric acid and creatinine in blood of SLE patient that mean (**6.59** ±0.22) (**0.95** ±0.06) ranged (3.40-11.30) (0.49-3.55) respectively, when compared to healthy control mean

(**4.55** ±0.16) (**0.60** ±0.02) ranged (2.90- 6.00)(0.38- 0.90) respectively. There was significant difference between uric acid and creatinine in SLE patients and healthy control $p < 0.05$, as shown in figure (4.16)(4.17).

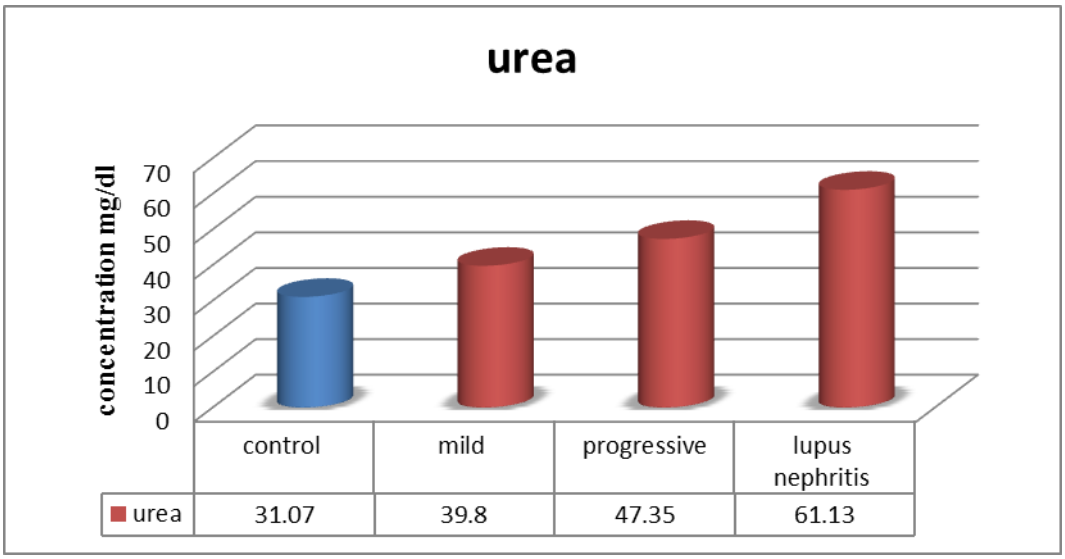


Figure 4. 12: Concentration of urea in SLE patients and healthy control.

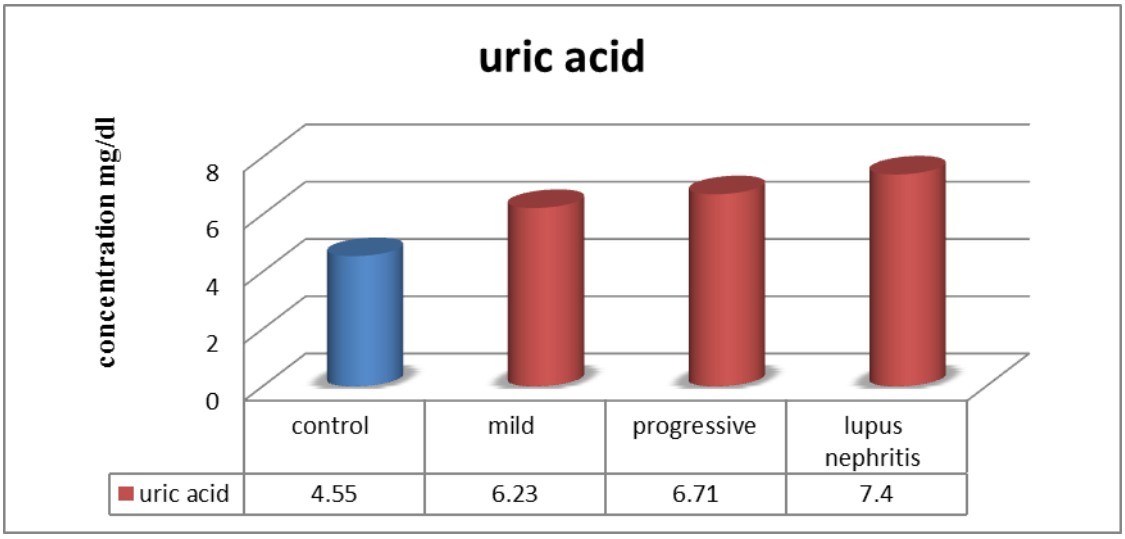


Figure 4. 13: Concentration of uric acid in SLE patients and healthy control.

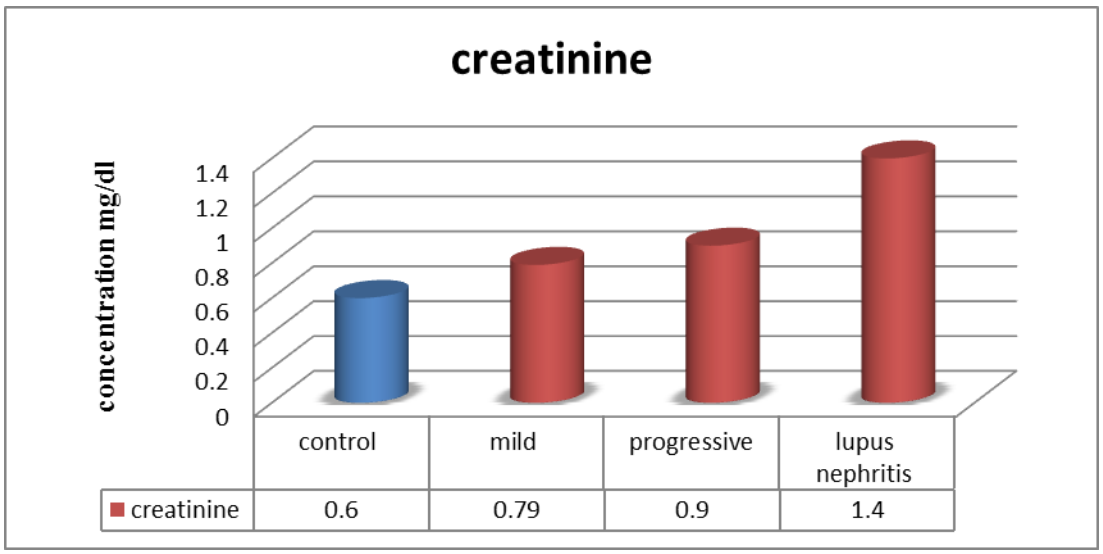


Figure 4. 17: Concentration of cearіatinine in SLE patients and healthy control.

In the kidney failure, machines are needed to cleaning the blood of accumulated waste products in a process called dialysis. This may explain why the concentration of urea , uric acid and creatinine in the blood of SLE patients increased (Parslow *et al.*, 2001and Waldman and Maldman, 2005).

This study appears to explore the association of serum urea, creatinine and uric acid with clinical and laboratory characteristics in SLE patients, independent of lupus renal involvement. Of particular interest, although these three components in serum are all common marker of renal function, they showed almost completely different associations with various clinical features in SLE patients.

Table 4. 2: Correlation between different parameters in patients with SLE.

Variables		ANA	ds-DNA	ESR	C3	C4
ANA	**r**	1	0.81**	0.42**	-0.43-**	-0.56-**
	p		0.00	0.00	0.00	0.00
ds-DNA	**r**	0. 81**	1	0.44**	-0.23-	-0.49-**
	p	0.00		0.00	0.06	0.00
ESR	**r**	0.42**	0.44**	1	-0.45-**	-0.26
	p	0.00	0.00		0.00	0.00

C3	**r**	-0.43-**	-0.23-	-0.45-**	1	0.47**
	p	0.00	0.04	0.00		0.00
C4	**r**	-0.56-**	-0.49-**	-0.26	0`.47**	1
	p	0.00	0.00	0.04	0.00	

r=Correlation Coefficient , p-value is significant at level 0.05

*** Correlation is significant at the 0.05 level , ** Correlation is significant at the 0.01 level**

Table 4.3: Correlation between autoantibodies and (cystatin C ,thymosin β4 and Vitamin D_3) in SLE patients

Variables		**Cystatin C**	**Thymosin β4**	**Vitamin D_3**
ANA	**r**	0.32*	-0.25-*	-0.52-**
	p	0.00	0.04	0.00
ds-DNA	**r**	0.47**	-0.25-**	-0.49-*
	p	0.00	0.01	0.00

r=Correlation Coefficient , p-value is significant at level 0.05

*** Correlation is significant at the 0.05 level , ** Correlation is significant at the 0.01 level**

4.6 Biochemical test deepened on Cys.C index.

The mean of cystatin C in SLE patients is (**3.37** ±0.11) rang (0.89-4.8) with kidney failure test urea , uric acid and ceriatinine (**82.9** ±3.69 ; **7.75** ±22 ; **1.59** ±0.06) rang (18.5-155 ; 3.4-11.3; 0.94-3.5) respectively, compared with healthy control (**0.6** ±0.03) rang (0.39-1.35), (**31.07** ±1.1 ; **4.55** ±0.16 ; **0.6** ± 0.02) rang (21-43 ; 2.9-6 ; 0.38-0.9) respectively, as shown in figure (4.18)(4.19)(4.20)(4.21).

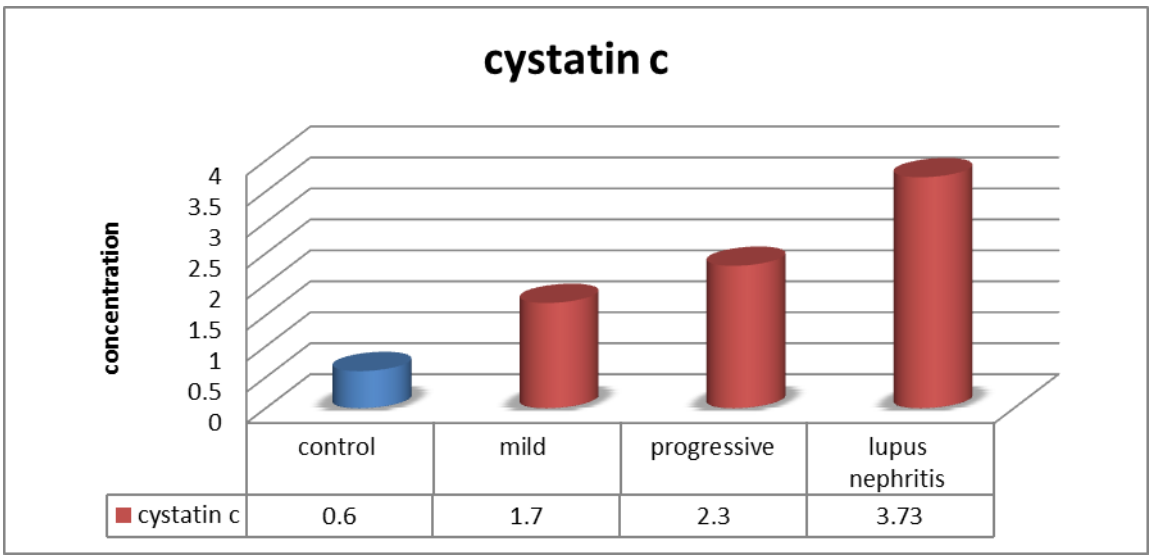


Figure 4. 18: Concentration of cystatin C in SLE patients and healthy control.

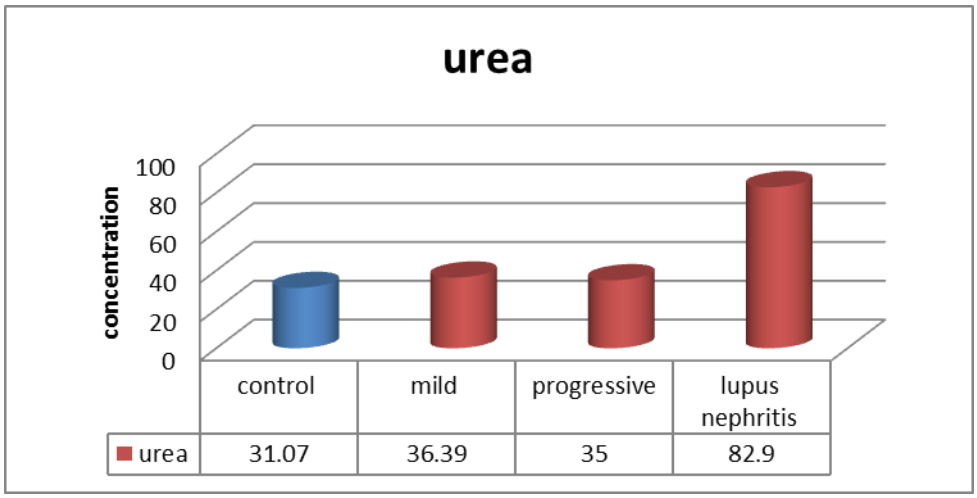


Figure 4. 19: Concentration of urea in SLE patients and healthy control.

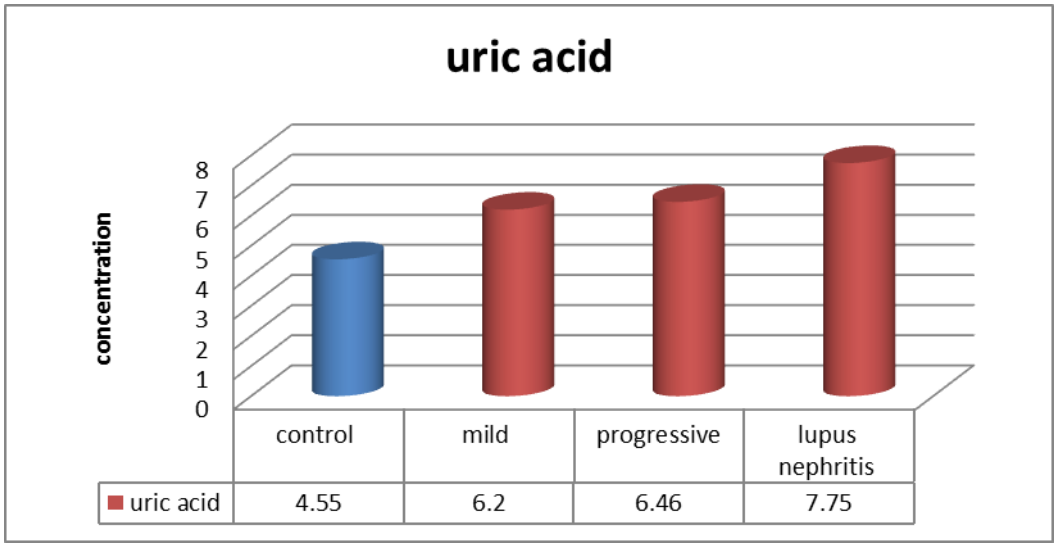


Figure 4. 20: Concentration of uric acid in SLE patients and healthy control.

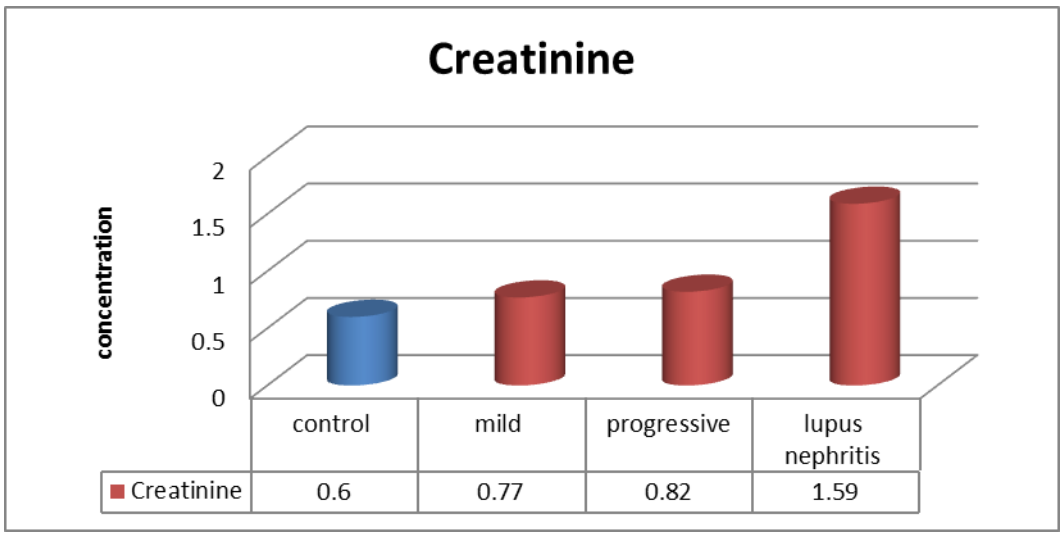


Figure 4. 14: Concentration of creatinine in SLE patients and healthy control

The result show that Cys C superior correlation coefficients values than serum Cr. With the accuracy of Cys C established, determination of its utility as a measure of GFR in clinical practice rests on its cost-effectiveness over Cr level (Vikas *et al.*, 2002).

Early investigations demonstrated that serum Cys-C was indeed a marker of GFR, at least as good as serum creatinine level in the populations investigated (Lffberg and Grubb, 1979; Simonsen *et al.*, 1985).

These studies also showed that the serum Cys-C concentration was a better GFR marker than the serum levels of the other low molecular mass proteins investigated, such as h2-microglobulin, retinol binding protein, and complement factor D (Bfkenkamp *et al.*, 2002).

The most commonly used laboratory parameter to estimate GFR is serum creatinine. The limitations of serum creatinine as an ideal marker of GFR in children and adolescents are well established. Creatinine production depends on muscle mass (Vinge *et al.*, 1999).

Conclusions

1. As the result showed on increasing concentration of ANA, ds-DNA and ESR, while, there were decreasing concentration of C3 and C4, so we can suggest that there is a relationship between there criteria and the flare up of disease.
2. Low concentration vitamin D_3 in all patients with systemic lupus erythematosus, especially in lupus nephritis. And treatment with Ca+/vitamin D_3 supplement, modulate the immune response of patients with SLE leading to improve in general condition of the patients, and induction a remission of the disease.
3. There is an inverse relationship between Thymosin β 4 and SLE disease activity index.
4. Cystatin C is a good marker for predicting the kidney failure at early stage of impaired GRF in SLE patients compared with routine kidney failure test.

Recommendations

1. Possibility for using vitamin D_3 as a treatment to modulate the immune response in patients with systemic lupus erythematosus, especially in lupus nephritis.
2. Further studies to use thymosin β 4 as a treatment to enhance the naive T-cell in the secondary immune organ for patients with systemic lupus erythematosus.
3. Draw attention about the using of cystatin C as predicting test for the kidney failure to detect the early stage of impaired GRF.
4. Further studies are needed to shed light on the importance of vitamin D3 on T-reg cells function.
5. More research on molecular study can be carried out to evaluate the genetic role on systemic lupus erythematosus.

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Appendix (5)

Figure (5 – 1) : Euroimmun 25-OH Vitamin D ELISA .

Figure (5 – 2) : thymosin β4 ELISA Kit.

Figure (5 – 3): Complement (C3,C4) Kits

Figure (5 – 4): Geneus /PA54

الخلاصة

الذئبة الحمراء الجهازية (مرض الذئبة الحمراء) هو مرض مزمن ويعد من امراض المناعة الذاتية الجهازية، يتميز مصليا كونه غير طبيعي من الناحية المناعية الخلوية والخلطيه والتي تتضمن الاجسام المضادة الذاتية المتعددة ضد انتجينات الاعضاء الداخلية المتخصصة .

هدفت هذه الدراسة الى الكشف عن بعض المعاملات المناعية والتي تشمل (anti-nuclear antibodies, anti-double strand DNA) باستخدام تقنية الاليزا ، وعوامل المتمم (C3 , C4) باستخدام طريقة نفينومتري، ذات الصلة بمرضى داء الذئبة الحمراء الجهازية ، والتي لها علاقة بمستوى الثايموسين β4 و وتركيز فيتامين D_3 ، بالإضافة الى دراسة الفحوصات الكيموحيوية والتي تشمل (السستاتين C، واليوريا، يورك اسيد والكرياتنين) .

وشملت هذه الدراسة 60 مريضا يعانون داء الذئبة الحمراء الجهازية ، ارتادوا مستشفى مدينة الامام الحسين الطبية (ع) ، و30 عينه من الاشخاص الاصحاء للمقارنة، في محافظة كربلاء ، خلال المدة من بداية كانون الثاني 2015 الى نهاية ايار 2016

كانت الغالبية العظمى من مرضى داء الذئبة الحمراء الجهازية من الاناث بنسبة (95%) ونسبة الذكور الى الاناث 1:19 . وكان متوسط اعمار مرضى داء الذئبة الحمراء الجهازية (1.53 ± 36.11) تراوحت بين (7 – 65) مقارنة مع مجموعة الاصحاء، كان متوسط اعمارهم (2.32 ± 37.56) تراوحت بين (17 – 65) لكلا الجنسين .

اظهرت النتائج زيادة في تركيز كل من الاجسام المضادة (ANA, ds-DNA) في المرضى الذين يعانون من التهاب الكلية الذئبة مقارنة مع مرضى داء الذئبة الحمراء الجهازية المتوسطة والمتقدمة بينما كانت عوامل بروتينات المتمم (C3,C4) في المصل اظهرت انخفاض معنوي في التركيز عند المرضى الذين يعانون من التهاب الكلية الذئبة بالمقارنة مع المتوسطة والمتقدمة .

علاوة على ذلك، اشارت النتائج الى وجود انخفاض معنوي في مستوى الثايموسين β4 في التهاب الكلية الذئبة بالمقارنة مع شكل داء الذئبة الحمراء الجهازية المتوسطة والمتقدمة .

اظهرت النتائج انخفاض معنوي في تركيز فيتامين د في مرضى التهاب الكلية الذئبة بالمقارنة مع المتوسطة والمتقدمة . من جهة اخرى اظهرت الدراسة ان العلاج بمكملات فيتامين د يلعب دورا مهم في التعديل المناعي لمرضى داء الذئبة الحمراء الجهازية

في النهاية سجلت نتائج الدراسة ان التهاب الكلية في مرضى داء الذئبة الحمراء الجهازية زيادة معنوية في تركيز السستاتين سي بالمقارنة مع المتوسطة والتقدمية.

ويمكن حساب النتيجة باستخدام مؤشر السستاتين سي الذي شمل ترتيب مرضى الذئبة الحمراء على اساس تركيز السستاتين سي.

وأظهرت النتائج ارتفاع معنوي في تركيز السستاتين سي في مؤشر السستاتين سي مقارنة مع SLEDAIفي مرضى الذئبة الحمراء، ووجد علاقة بين نتائج تركيز السستاتين سي ، واليوريا والكرياتينين في التهاب الكلية الذئبة.

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دراسة مناعية في مرضى الذئبة الحمراء الجهازية الذين يشكون من الفشل الكلوي المزمن

رسالة مقدمة
إلى مجلس كلية العلوم - جامعـــــة كـربـــــلاء
وهي جزء من متطلبات نيل درجة الماجستير
في علــوم الـحيــاة

مقدمة من
نور هاشم ماشاء الله الهاشمي
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