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College of Applied Medical Sciences

Department of Clinical Analysis

Study of Some Immunological and Biochemical Parameters in Females Infected with Lupus Arthritis

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Written by

(Mohammed Rabie Nasih)

B.Sc. Medical Laboratory Science Technology
AL-Mustafa University College, 2018

Supervised by
Prof. Dr. Hassan Ali Hussein

Assist.Prof.Dr. Radhwan Mohammed Hussein

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بِسْمِ اللّهِ الرَّحْمَنِ الرَّحِيم

(قَالُواْ سُبْحَٰنَكَ لَا عِلْمَ لَنَآ إِلَّا مَا عَلَّمْتَنَآۖ إِنَّكَ أَنتَ ٱلْعَلِيمُ ٱلْحَكِيمُ)

صدقَ اللّهُ العلي العظيمُ

سورة البقرة الآية ﴿٣٢﴾

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Signature

Prof.Dr.Hassan Ali Hussein Al-Saadi

Supervisor

Date: / / 2023

Signature

Assist.Prof.Dr. Radhwan Mohammed Hussein

Supervisor

Date: / / 2023

Head of Department Recommendation

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

Signature

Assist. Prof. Dr. Linda Hameed Turki

Head of the Department of Clinical Laboratories

College of Applied Medical Sciences

University of Kerbala

Date: / /2023

Approval Certification

We certify that the thesis entitled **"Study of Biochemical Changes in Female with Lupus Arthritis"** fulfills partial requirements of the degree of Master in Clinical Laboratories.

Signature

Head of Clinical Laboratories Department

Assist. Prof. Dr. Linda Hameed Turki

College of Applied Medical Sciences

University of Kerbala

\ \ 2023

Signature

Vice Dean Scientific Affairs

Assist. Prof. Dr. Huda Abdalreda Abdullah

College of Applied Medical Sciences

University of Kerbala

21\9\ 2023

Committee Certification

We, the examining committee, certify that we have read the thesis entitled **" Study of Some Immunological and Biochemical Parameters in Females Infected with Lupus Arthritis"** and have examined the student **(Mohammed Rabie Nasih)** in its content and that in our opinion it is accepted as a thesis for degree of Master of **Clinical Laboratories.**

Signature

Prof. Dr. Wafaa Sadiq AL-Wazni

(Chairman)

/ / 2023

Signature

Prof. Dr. Rana Fadhil Mousa

(Member)

/ / 2023

Signature

Prof. Dr. Dunya Fareed Salloom

(Member)

/ / 2023

Signature

Prof. Dr. Hassan Ali Hussein

(Member& Supervisor)

/ / 2023

Signature

Assist.Prof.Dr. Radhwan Mohammed Hussein

(Member& Supervisor)

/ / 2023

I have certified upon the discussion of the examining committee .

Signature

Prof. Dr. Jawdat Noori Gaaib

Dean of the College of Applied Medical Sciences / University of Kerbala

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Mohammed 2023

Dedication

To …Martyrs of Iraq

To... the person we take as a role model, my dear father

To...the source of success and success in this world, my dear mother

To... my creative brothers all

To... my dear wife

To... the delight of the eyes and the pleasure of my liver (Mustafa and Haneen)

To every patient who is suffering from SLE and need our efforts

Special thanks to my close colleague...

Mohammed 2023

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Abbreviations

Abbreviation	*Meaning*
(1,25OH) Vit-D	1,25-Dihydroxyvitamin D
ACR	American College of Rheumatology
anti-dsDNA	Anti-Double Strand Deoxyribonucleic Acid
AHAs	Anti-Histone Antibodies
ANA	Anti-Nuclear Antibody
RibP	Anti-Ribosomal P
Anti-sma	Anti-Smith Antigen
BUN	Blood Urea Nitrogen
BMI	Body Mass Index
BMD	Bone Mineral Density
CNS	Central Nerve System
Cen-ANA	Centromere
CVA	Cerebrovascular
CD40	Cluster Of Differentiation
CBC	Complete Blood Count
C-ANA	Cytoplasmic
C1q	Component 1q
C2	Component 2
C3	Component 3
ELISA	Enzyme Linked Immune Assay
ESR	Erythrocyte Sedimentation Rate
EDTA	Ethylene Diamine Tetra Acid
EULAR	European League Against Rheumatism
Fc	Fragment Crystallizable
H-ANA	Homogenous
HM-ANA	Homogenous + Mitochondrial
HN-ANA	Homogenous + Nucleolar
HRP	Horseradish Peroxidase

HLA	Human Leukocyte Antigen
IC	Immune Complexes
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IFN	Interferon
IL-1	Interleukin 1
kD	Kilodalton
kg	Kilogram
µL	Microliter
ml	Mili Later
min	Minutes
M-ANA	Mitochondrial
ng	Nanogram
nm	Nanometer
NK	Natural Killer
NETs	Neutrophil Extracellular Traps
NPN	Non-Protein Nitrogenous
N-ANA	Nucleolar
OD	Optical Density
OC	Osteocalcin
OPG	Osteoprotegerin
PTH	Parathyroid Hormone
PBS	Phosphate Buffer Saline
pDC	Plasmacytoid Dendritic Cells
pH	Potency Hydrogen
PRL	Prolactin
ROC	Receiver Operating Characteristic
RANKL	Receptor Activator of The Nuclear Factor-KB Ligand
RBC	Red Blood Cell
RA	Rheumatoid Arthritis

RNA	Ribonucleic Acid
rpm	Rotation Per Minute
S.Cr	Serum Creatinine
S-ANA	Speckled
SC-ANA	Speckled + Cytoplasmic
SD	Standard Deviation
SPSS	Statistical Package for Social Science
SLE	Systemic Lupus Erythematosus
TMB	Tetramethylbenzidine
TBS	Tris Buffered Saline
TNF	Tumor Necrosis Factor
TNFSF	Tumor Necrosis Factor Superfamily
UV	Ultraviolets
UVB	Ultraviolets B
UK	United Kingdom
USA	United States of America
VDR	Vitamin D Receptor
β-CTX	B-Carboxy-Terminal Cross-Linking
Wnt	Wingless Int-1

Summary

Lupus arthritis is a synovitis that affects two or more joints and is characterized by swelling, effusion, or pain in two or more joints that lasts at least 30 minutes in the morning. Up to 90% of SLE patients will experience musculoskeletal involvement, making it one of the condition's most prevalent symptoms.

This study investigated the serum level of Osteoprotegerin (OPG), β-crosslaps (β-CTX), Osteocalcin (OC) and Vitamin D3 (Vit-D3) in patient with lupus arthritis. Measure the effect of disease severity on the serum levels of (OPG, β-CTX, OC and Vit-D3). To assess the specificity and sensitivity, cut off value of the factors. Evaluated the correlation of these factorion with Age, Body mass index, anti-nuclear antibody, Anti-double strand deoxyribonucleic acid (dsDNA), C3, C4, CBC, ESR, $S.Ca^{+}$, B.Urea and S.Cr.

This case-control study was conducted on 131 SLE women and 50 healthy individuals over a period of 4 months, from November 2022 until March 2023. SLE women included in this study were collected from Baghdad hospital in Medical City, Baghdad governor. The practical part occurred at the research laboratories of the International Center for Research and Development located in the city of Kadhimiya and the teaching laboratories in the Medical City. All SLE women who were included in the study were subjected to physical examination, age, BMI, and laboratory investigations, including urine examination, ANA, ds-DNA, BUN, S.Cr, ESR, dsDNA, C3, C4, and $S.Ca^{+}$. Clinical examinations were conducted depending on the specialty of the consultant doctor, and they were categorized according to the Roma Helper program into three groups (inactive N=30, mild & moderate N=49 and severe N=52) according to the disease severity.

Results of the OPG and β-CTX levels were significantly increased in lupus arthritis while OC and Vit-D3 levels were significantly decreased. The OPG and β-CTX levels were positive correlation with disease activity while OC and Vit-D3 levels were negative correlation with disease activity. β-CTX showed excellent diagnostic performances for lupus arthritis where results shown AUC (0.99), sensitivity (0.99), specificity (0.70), cut-off points (905.78) and ($p < 0.001$). The correlation of OPG with ANA, ESR, S,Cr and Vit-D3 where positive correlation while OPG with β-CTX were negative correlation. The correlation of OC with C3, Age, HB, β-CTX, OPG where positive correlation while OC with BMI was negative correlation. The result of Vit-D3 were positive correlation with RBC and β-CTX, While negative correlation with C3 and HB. The result of β-CTX were positive correlation with S.Cr, BUN, ESR, WBC, RBC, OC, Ca^{+} and Vit-D3,While negative correlation with OPG and ANA. In conclusion the OPG and β-CTX levels were significantly increased in lupus arthritis while OC and Vit-D3 levels were significantly decreased. In conclusion β-CTX was good indicator for severity of lupus arthritis.

Chapter one

Introduction

1.Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune systemic disease with a wide range of clinical manifestations that predominantly affects women (Zucchi *et al*., 2022). SLE characterized by aberrant activity of the immune system (Kiriakidou and Ching, 2020). The underlying causes of SLE are incompletely understood, but include both genetic and environmental risk factors, which resulted in a significantly change in immune environment commonly characterized by reduced regulatory T cells, increased effector T cells and enhanced B cell activation (Legorreta-Haquet *et al*., 2016) (Novak, 2000). The causes of SLE in people who are genetically vulnerable by exposure to environmental factors including UV radiation, viruses, and toxins which causes a loss of immunological tolerance and an abnormal activation of the autoimmune system (Ameer *et al.,* 2022).

Common symptoms of SLE include painful and swollen joints, fever, chest pain, hair loss, mouth ulcers, swollen lymph nodes, feeling tired, and a red rash which is most commonly on the face (Kiriakidou and Ching, 2020). Often there are periods of illness, called flares, and periods of remission during which there are few symptoms (Novak, 2000).

lupus arthritis is a symmetric polyarthritis that preferentially involves the small joints over the large joints although any joint may be affected. The duration of the joint symptoms is variable, ranging from hours to weeks to months. Swelling of the joint as a consequence of joint fluid or synovial proliferation can be present although the swelling is often not as prominent as it is with rheumatoid arthritis (RA). Physical examination may reveal tender joints without swelling. Other associated signs and symptoms of lupus arthritis include joint erythema, pain ranged decrease motion of the joint to morning stiffness, which was present in 67% in one

study. The most common joints affected are the hand joints as well as the knees. Shoulders, ankles and elbows are less commonly affected but can be involved (Grossman, 2009).

Osteoprotegerin (OPG) is a glycoprotein that belongs to the tumor necrosis factor superfamily (TNFSF), The molecular weight of OPG, which is 60 kD, is made up of 401 amino acids. OPG is primarily recognized as a regulator of bone remodeling in a variety of clinical contexts and physiological situations (Dutka *et al*., 2022). OPG is produced by a variety of organs and tissues (Kiani *et al.,* 2017). OPG is known as a decoy receptor for receptor activator of the nuclear factor-κB ligand (RANKL) which prevents RANKL-RANK binding (RANKL inhibitor) (Tobeiha *et al*., 2020). OPG competes with RANKL for RANK, OPG is regarded as a direct activator of osteoclast functions in addition to being a soluble RANKL decoy receptor (Ma *et al*., 2022). Several studies showed increase serum OPG in SLE patients compare than healthy control (Park *et al.,* 2014) (Kwok *et al*., 2009).

Type I collagen telopeptide for the amino (NTx) and carboxyl (CTx) termini are also released upon degradation of Type I collagen by osteoclasts (Konukoglu, 2019). Newly synthesized collagen Type I comprises non isomerized C-telopeptide (αCTX), but with bone matrix maturation αCTX is converted to its isomerized β form (βCTX) (Garnero *et al*., 2009). The β-CTx are chief element (∼90%) of the protein matrix of bone. β-CTx is released into the blood during bone resorption and is excreted mainly by the kidneys. Its estimation serves as a specific marker for the degradation of mature type I collagen from bone (Mohamed, 2019). Osteoclasts dissolve bone matrix by secreting proteolytic enzymes and then produce β -CTX, which is also used to evaluate the activity of osteoclasts (Zhu *et al*., 2021). β -CTX act as bone resorption marker are elevated in SLE patients and positively associated

with SLE disease activity in several studies (Bogaczewicz *et al*., 2015) (Zhu *et al*., 2021) (Guo *et al*., 2017).

Osteocalcin (OC) a bone protein is non-collagenic, which binds calcium and phosphate, two indicators of dynamic bone forming activity (Sunarya *et al*., 2022). OC is released by osteoblasts during bone formation and binds with the mineralized bone matrix (Hlaing and Compston, 2014).

There are two forms of osteocalcin, the first is Carboxylated form are involved in calcium and hydroxyapatite binding, allowing osteocalcin deposition in mineralized bone matrix. On the contrary, non carboxylated form osteocalcin has a low affinity for hydroxyapatite and is more easily released into the circulation. However, both the carboxylated and the undercarboxylated forms are detectable in the peripheral blood, as well as total osteocalcin that is usually measured as a marker of bone formation (Patti *et al*., 2013). Several studies showed decrease level of OC and negatively associated with disease activity (Baker-LePain *et al*., 2011) (Guo *et al*., 2017).

Vitamin D, also known as calciferol, comprises a group of fat-soluble seco-sterols. (Ross *et al*., 2011). Vitamin D, as a steroid hormone, exhibits regulatory effects on growth, proliferation, apoptosis and function of the immune system cells that are associated with pathophysiology of SLE (Hassanalilou *et al*., 2018). In recent decades, interest in vitamin D has increased exponentially, particularly as a vitamin D deficit has been associated with multiple diseases and globally, there appears to be a high vitamin D deficiency. Currently, the role of vitamin D as a hormone has been confirmed in numerous physiological and pathophysiological processes, related to various organs and systems of the human body (Dominguez *et al*., 2021). The prevalence of vitamin D deficiency is high and evidence shows that

it can contribute to the morbidity and mortality of numerous chronic diseases, including SLE (Hassanalilou *et al*., 2018).

Aim of Study

Evaluate some immunological and biochemical markers in women with lupus arthritis for the purpose of identifying as indicted for the development of this disease

The main objectives of present study can be summarized as:

1- Ten milliliters (ml) of venous blood were withdrawn from lupus arthritis patients and healthy individuals
2- Evaluate the serum level of OPG, BCTX, OC and Vit-D3 in patient with lupus arthritis.
3- Determine the effect of disease severity on the serum levels of (OPG, BCTX, OC and Vit-D3).
4- Evaluate the correlation of these factorion with (Age, BMI, ANA, dsDNA, C3, C4, CBC, ESR, $S.Ca^{+}$, BUN and S.Cr).

Chapter Two

Literature Review

2.1. Systemic Lupus Erythematosus (SLE):

Systemic Lupus Erythematosus is autoimmune disease characteristic of aberrant activity of the immune system (Kiriakidou and Ching, 2020). SLE clinically heterogeneous disease in origin, which characterized by the presence of autoantibodies directed against nuclear antigens (Manson and Rahman, 2006). SLE can affect skin, joints, kidneys, lungs, nervous system, serous membranes and/or other organs of the body(Ismail Zoair *et al*., 2021). The causes of SLE are vague, but it may be due to infection, environmental factors involve UV radiation, demethylating treatments, infectious or endogenous viruses (Bertsias *et al*., 2012), antibiotics (particularly those from the penicillin and sulpha families), infections and hormones (Carey *et al*., 2008), (Lisnevskaia *et al*., 2014).

According to the definition of Arthritis, it is a synovitis that affects two or more joints and is characterized by swelling, effusion, or pain in two or more joints that lasts at least 30 minutes in the morning. (Ceccarelli *et al*., 2022). Lupus arthritis is typically a non-erosive, symmetrical inflammatory polyarthritis affecting predominantly the small joints of the hands, knees, and wrists, although any joint can be involved (Mukkera *et al*., 2022). up to 90% of SLE patients will experience musculoskeletal involvement, making it one of the condition's most prevalent symptoms. (Cervera *et al*., 2003), It is recorded in up to 60% of illness flares and acts as the first symptom in 60-80% of cases. (Mosca *et al*., 2019). joint complaints like arthralgia or arthritis are frequently temporary, they may resemble RA and present with persistent pain, swelling, stiffness, and disability. The wrist, knee, and hand joints are the most commonly affected, however tenosynovitis or tendonitis may also be present (Ceccarelli *et al*., 2022).

Patients with SLE are at increased risk for osteoporosis for many reasons. First, the glucocorticoid medications often prescribed to treat SLE can trigger significant bone loss, Second, chronic inflammation, Third, pain and fatigue caused by the disease can result in inactivity, further increasing osteoporosis risk, these factors have become more prominent (Garcia-Carrasco *et al*., 2009).

Musculoskeletal is common both at presentation and during the course of SLE. the main features are arthritis, arthralgia, and/or tenosynovitis. The arthritis is painful and the symmetric polyarthritis affecting both large and small joints and morning stiffness is also common. because more than 90% of patients who will develop arthritis have developed the arthritis within the first year of diagnosis (frequently at diagnosis). Although myalgia is seen in 20% to 30% of patients, true myositis occurs much less frequently, but when present. Noninflammatory Musculoskeletal pain frequently occurs following treatment and may be the result of a pain amplification syndrome secondary to glucocorticoid therapy(Silverman and Eddy, 2010).

2.2 History of SLE:

Moriz Kaposi (1837–1920) was credited with being the first doctor to describe internal organ involvement in LE in 1872. He also came up with the acronym SLE to distinguish it from LE (Lupus erythematosus), a skin-only condition. LE classified with internal organ involvement as Lupus erythematosus disseminates aggregates SLE. LE patients with fever, weight loss, anemia, amenorrhea, dysmenorrheal, adenitis, arthralgia/arthritis, increased mental disturbance, and coma, realizing many major clinical features of SLE. Sir William Osler is credited for the implication of lupus nephritis (1849-1919) as the cause of early demise in SLE due to his description of two cases of fatal nephritis that developed shortly after the appearance of the skin disease. Prior to the description of fatal nephritis, the causes of death in LE were assumed to be due to infections. Regarding his contribution to the study of

SLE, it has been over-emphasized, in his description of 29 cases of SLE from 1895 to 1904, only two were clearly SLE with the majority of the cases being Henoch-Schonlein purpura (Fua and Gaskind, 2020).

2.3: Epidemiology

According to estimates, there are 0.4 million newly diagnosed persons each year and an incidence of systemic lupus erythematosus of 5.14 per 100,000 individuals worldwide. The estimates for men were 1.53 per 100 000 person-years and 0.06 million people yearly, while they were 8.82 for women and 0.34 million for women, respectively. The greatest estimates of SLE incidence were in Barbados, the United States, and Poland. According to estimates, there are 3.41 million people affected by SLE worldwide, with a prevalence of 43.7 per 100,000 people. The estimations for men were 9.26 per 100 000 people and 0.36 million people, compared to women's values of 78.73 per 100 000 people and 3.04 million people.(Tian *et al*., 2023).

With a 9:1 female to male ratio, it is more common in women of childbearing age (Mukkera *et al*., 2022). SLE is more common in women than in men a study was according on this trend and that was the result X-linked anomaly it constitutes Xi gene expression in man SLE B cells indicate significance contribution to X-linked gene expression on female bias in SLE (Zucchi *et al*., 2022).

2.4: Pathogenesis of SLE

A complicated interplay between the genome and the environmental influence results in an epigenetic alteration that affects the expression of particular genes involved in the pathogenesis of SLE. in people who are genetically susceptible, Exposure to environmental variables causes a loss of immunological

tolerance and causes an abnormal activation of the autoimmune system. (Ameer *et al*., 2022a).

Loss of immune tolerance, defective B cell suppression, and the shifting of T helper 1 (Th1) toTh2 immune responses leads to B cell hyperactivity and the production of pathogenic autoantibodies (Mok and Lau, 2003). 53–95% of SLE patients have complications with their musculoskeletal system (Bertsias *et al*., 2012).

In SLE patients, the immune system releases important nuclear self-antigens in response to changes in cell death pathways, including apoptosis. (Mahajan *et al*., 2016) as well as through the neutrophil specific death releasing neutrophil extracellular traps (Lood *et al*., 2016), and accumulate up as a result of the poor removal of necrotic cell-derived debris (Nagata *et al*., 2010). Restrictive human leukocyte antigen (HLA) are used to exhibit these self-antigens (Deng and Tsao, 2010) by follicular dendritic cells (Blanco *et al*., 2001) to autoreactive B cells in germinal centers of secondary lymphoid organs, and activate the differentiation and clonal expansion of CD4+ autoreactive T cells. Activated T helper cells release interferon (IFN)-gamma, and subsequently mature dendritic cells release pro-inflammatory cytokines such as interleukin 1 (IL-1) and tumor necrosis factor (TNF), and activate B cells (Blanco *et al*., 2001). IL4 play a role in pathogenesis of SLE (Al-Saadi, 2015). Other study suggested the critical role of Interleukins (4,10,18 and 35) inactivation and the regulation of immune responses in patients suffering SLE. IL-4 and immunoregulatory IL- 10 contribute to B cell activation. IL-18 as a biomarker in distinguishing SLE in prognosis early indicator SLE development in lupus nephritis.IL-35 as biomarker detector for SLE activity. These indicators provide us renal disorder impression of SLE(Alwandawi and Al-Saadi).

Immune complexes containing nuclear self-antigens deposit or form in situ in the tissues, activate complement, and lead to tissue destruction as one important stage in the pathogenesis of SLE (Mohan and Putterman, 2015). Through somatic hypermutation and affinity maturation, the immune response shifts from low-affinity immunoglobulin M (IgM) to highly specific high-affinity IgG auto-antibodies targeted toward more constrained epitopes of the self-antigens (Fraser *et al*., 2003), as shown (Figure 2-1).

Osteoimmunology studies indicate a complex interplay between the immune and skeleton systems. Although osteoporosis and high fracture risk are well-known consequences of SLE, the cause of low bone mass in SLE patients remains unclear. The deposition of immune complexes (ICs) plays a major role in the pathogenesis of SLE. Receptors for the Fc of IgG are important for IC clearance. In SLE, impaired Fc-mediated IC clearance initiates the release of inflammatory mediators and influx of inflammatory cells(Visitchanakun *et al*., 2019).

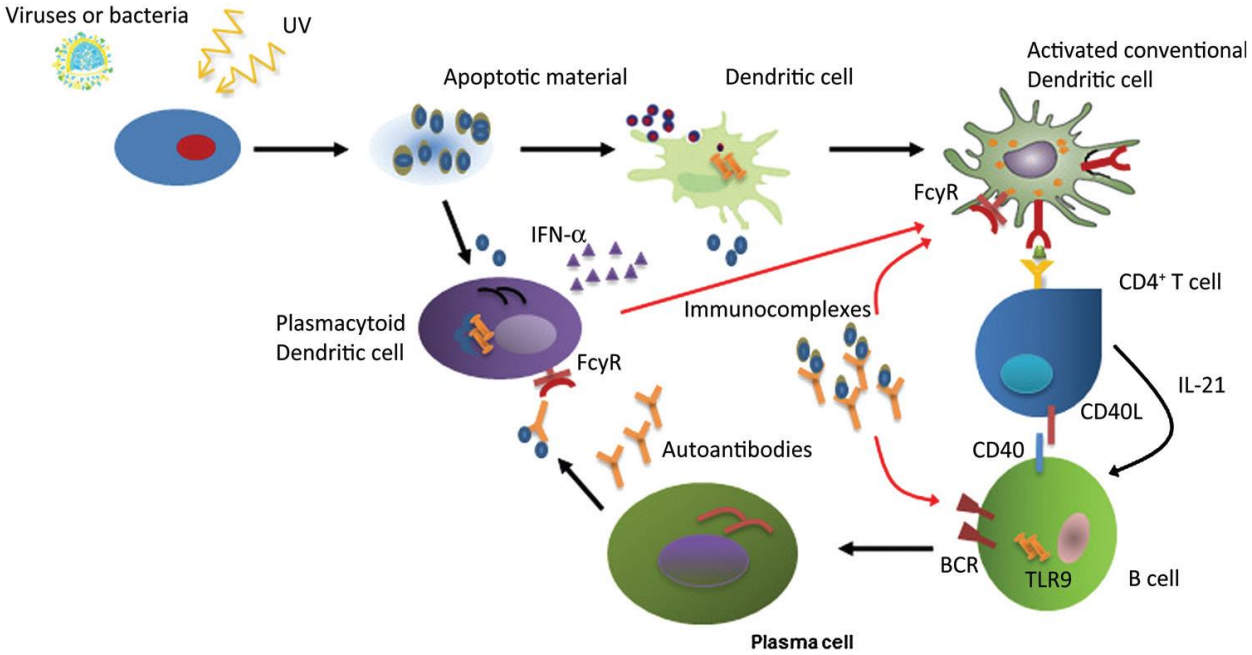


Figure (2-1): Differentiation and formation of autoantibody producing plasma cell.(Bertsias et al., 2012)

2.5. Immunological Abnormalities of SLE

SLE is characterized by immune dysregulation involving both the innate and adaptive immune systems and all effector mechanisms have been shown to be defective. Silverman and Eddy suggested regarding loss of tolerance in SLE that one or more of the following factors play a role: First the generation of self-antigens on cell surfaces following apoptosis. Second abnormalities of innate immunity including Toll-like receptors; Third abnormalities of all arms of the adaptive immune system including Antigen -presenting cells, T cells, and B cells; Fourth epigenetics and, most recently, abnormal regulation of interferon-α (Silverman and Eddy, 2010).

2.6. Symptoms of SLE

Although lupus can affect any part of the body, most people experience symptoms in only a few organs. Table (2-1) lists the most common symptoms of people with lupus (Carey *et al*., 2008).

Table (2-1): Table of symptoms according to the Lupus Foundation of America(Carey *et al*., 2008)

Symptoms	Occurrence
arthralgia	95%
Fever more than 100 degrees F (38 degrees C)	90%
Arthritis	90%
Prolonged or extreme fatigue	81%
Skin rashes	74%
Anemia	71%
Kidney involvement	50%
Pain in the chest on deep breathing (pleurisy)	45%
Butterfly-shaped rash across the cheeks and nose	42%
Sun or light sensitivity (photosensitivity)	30%
Hair loss	27%
Abnormal blood-clotting problems	20%
Raynaud's phenomenon (fingers turning white and/or blue in the cold)	17%
Seizures	15%
Mouth or nose ulcers	12%

2.7.Etiology:

It is clear that the etiology of the disease is unknown but been has shown to result from complex multifactorial interactions between genetic, hormonal and environmental factors that lead to loss of self-tolerance (Vollenhoven and Arnaud, 2018). The immune system in SLE release the nuclear self-antigens with regard to alterations of cell death pathways, also apoptosis (Mahajan *et al*., 2016) and accumulates as a result of a defect in the removal of necrotic material derived from cells.Where the body, in order to achieve organic balance, swallows dead phagocytic cells, which are recognized because of the "eat me" feature on their surface. The dead cells are then transferred to lysosomes, to degrade their cellular components for reuse, inefficient engulfment of dead cells activates the immune system, causing disease such as systemic lupus erythematosus (Nagata *et al*., 2010).

2.7.1. Human Leukocyte Antigens (HLA)

In Human, Major Histocompatibility Complex known as HLA. The HLA grouped into three subclasses regions: the class I region, the class II region, and the class III region. There are thousands of polymorphic HLAs, many of them are proven to have correlations with diseases such as SLE (Pradana *et al*., 2020).

The relationship between HLA and SLE is complex, with different alleles and haplotypes at risk that have been reported across clinical and laboratory profiles. In addition, TNF-related genes and complement system proteins, which are also found in the HLA area, are highly related to SLE (Bang *et al*., 2016).

2.7.2. Genetic Factors:

SLE Patients have many clinical symptoms that are affected by the mutation of several genes, Polymorphisms in the genes that encode regions of the MHC

represent one of the most important risk factors for the development of SLE, especially HLA-DRB1 and HLADQB1(Bastidas Goyes *et al*., 2021).

2.7.3. Complement System:

Complement deficiencies within the classical pathway (C1q, C4, C3 and C2) of activation predispose for development of the autoimmune disease SLE. The association between complement deficiencies and SLE could be explained by several mechanisms, including impaired clearance of immune complexes and impaired handling of apoptotic cells, aberrant tolerance induction or changes in cytokine regulation. Also, during SLE disease flares, the complement system is activated giving rise to partial deficiency or dysfunction due to consumption. On the other hand, complement also takes part in the inflammatory reaction in the disease that gives rise to the tissue and organ damage (Truedsson *et al*., 2007). So, there was a positive relationship between C4 with anti-dsDNA in the SLE patients (Al-Saadi, 2015).

2.7.4.The Influence of the Environment:

The environmental elements may interact with a genetic predisposition to cause SLE. SLE has been associated with a number of environmental factors, including ultraviolet (UV), Cigarettes, silica, solvents, and infections, but none of these factors have been identified as direct causes of the disease (Vollenhoven and Arnaud, 2018).

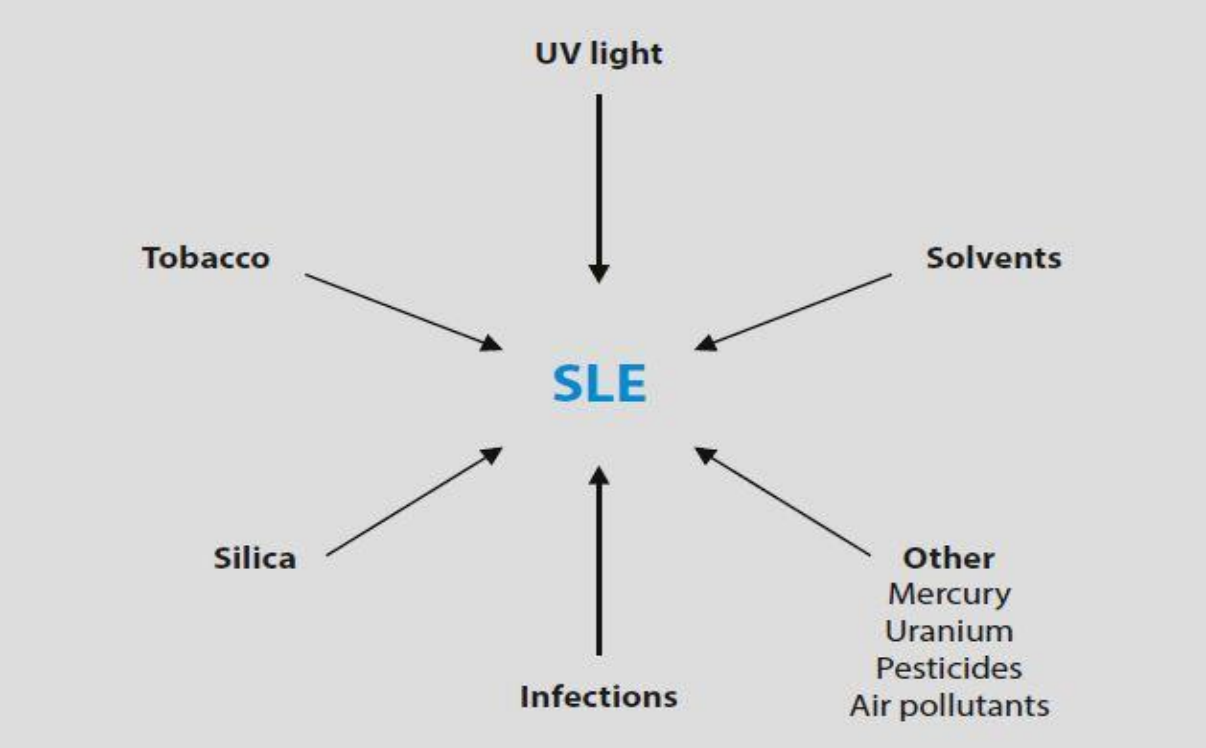


Figure (2-2): Environmental factor that many trig gory SLE (Vollenhoven and Arnaud, 2018).

2.7.4.1. Ultraviolet Light Exposure

One of the environmental elements that has been suggested and researched in relation to SLE is UV radiation exposure. Although it is well known that exposure to UV radiation may aggravate SLE that already exists, it is still unknown whether UV radiation exposure carries a risk of developing SLE (Barbhaiya and Costenbader, 2014).

2.7.4.2. The Role of Infections:

In people with SLE, infection is the most frequent type of pulmonary involvement. 20–55% of all deaths in SLE patients are caused by infections (Bertsias *et al*., 2012).

A broad spectrum of infections has been reported in SLE, including bacterial, mycobacterial, viral, fungal, and parasitic infections, with the respiratory, urinary

tract, and Central Nerve System (CNS) the most commonly involved sites. Risk factors for infections include increased clinical and/or serological SLE activity at baseline, major organ involvement (especially renal and lung) (Bertsias *et al*., 2012).

Through molecular mimicry or because the innate immune responses generated by viral may share pathogenic pathways with those generated by nuclear auto-antigens, infections may act as environmental triggers for SLE (Vollenhoven and Arnaud, 2018).

One theory for the etiology of SLE involves the virus's parvovirus B19, Epstein-Barr virus, human herpes viruses and cytomegalovirus (CMV). According to several research, SLE patients experience seropositivity or viremia more frequently than controls (Ulff-Møller *et al*., 2010).

2.7.4.3.The Influence of Hormones

The significant increase in risk seen in women strongly suggests a role of sex hormones in SLE; sex hormones are well-established regulators of the immune system, leading to the hypothesis that they play a role in SLE pathophysiology, Estrogen-containing contraceptives increase the risk of developing SLE (Gonzalez, 2020).

Sex hormones play vital roles in patients with SLE (including estrogens, progesterone, androgens, PRL) affecting the susceptibility and development of SLE. Some sex hormones (e.g., estrogens, PRL) may act as risk factors for SLE, and some (e.g., progesterone, androgens) may keep protective effects in the development of SLE (Pan *et al*., 2019). The levels of serum sex hormones manifested a decreased level of testosterone and increased prolactin in SLE patients when compared with the control group (Dong *et al*., 2022).

2.8: Diagnosis:

There is not a single laboratory test available right now that can tell if someone has SLE or not. The American College of Rheumatology (ACR) published a list of 11 symptoms or indicators that can identify SLE from other diseases in 1982 to aid doctors in the diagnosis of the disease. To suspect SLE, a person must have four or more of these symptoms (Table 2-2). not all symptoms occur at the same time.(Carey *et al*., 2008).

Table (2-2): The America- rheumatism association revised criteria for SLE. (Carey *et al*., 2008)

Features	**Characteristics**
Malar rash	Fixed erythema, flat or raised, sparing the nasolabial folds
Discoid rash	Erythematous raised patches with adherent keratotic scarring
Photosensitivity	Skin rash as a result of unusual reaction to sunlight
Oral ulcers	Oral or nasopharyngeal ulceration; may be painless
Arthritis	Non-erosive, involving two or more peripheral joints
Serositis	a. Pleuritis (convincing history of pleuritic pain, rub or pleural effusion) or b. Pericarditis (rub, ECG evidence or effusion
Renal disorder	a. Persistent proteinuria >0.5 g/day, or b. Cellular casts (red cell, granular or tubular)
Neurological disorder	Seizures or psychosis in the absence of offending drugs or metabolic derangement
Haematological disorder	a. Haemolytic anemia or b. Leucopenia (<4000/mm3) c. Lymphopenia (<1500/mm3) or d. Thrombocytopenia (<100 000/mm3)
Immunology disorder	a. Anti-DNA antibodies in abnormal titer or b. Presence of antibody to Sm antigen or c. Positive antiphospholipid antibodies in the absence of offending drugs
Antinuclear antibody disorder	Abnormal titer of ANA
1. For the identifying patients for clinical studies, a person must have SLE if any four out of 11 are present serially or simultaneously. 2. On two separate occasions	

2.8.1. Autoantibodies

2.8.1.1. Anti-Nuclear Antibody (ANA)

The word "ANA" refers to a variety of autoantibodies that target any type of nuclear component, including proteins, nucleic acids (such as DNA or RNA), and protein-NA complexes. However, because it has a wider definition, the term "ANAs" is also used to refer to autoantibodies that target substances other than those found in the nucleus, particularly those that target cytoplasmic proteins like anti-ribosomal P (RibP). Furthermore, ANAs are distinct entities whatever of the antigenic target because they share some common characteristics and may have overlapping expressions. As a result, the primary test to describe ANAs by assessing their positivity. Characterizing ANAs titers and antigenic targets is predictive of the clinical symptoms of the disease, its pathogenicity, and its major site of activity in addition to being thought of as key markers in SLE. In SLE, anti-ds DNAs, a subclass of anti-DNA ANAs that bind double-stranded DNA, have a higher diagnostic value, and their titers have long been noted to signal the disease activity (Al-Mughales, 2022).

The different ANA patterns are abbreviated as follows: Homogenous (H-ANA), Speckled (S-ANA), Nucleolar (N-ANA), Cytoplasmic (C-ANA), Centromere (Cen-ANA), Mitochondrial (M-ANA), and some mixed patterns such as Speckled + Cytoplasmic (SC-ANA), Homogenous + Mitochondrial (HM-ANA), Homogenous + Nucleolar (HN-ANA) (Alsubki *et al*., 2020).

ANA consisting of diverse autoantibodies targeting nuclear and cytoplasmic cell components, is the serological hallmark of SLE and is present in 95–99% of SLE patients. Given the rarity of ANA-negative patients with SLE, detection of ANA is critical to SLE diagnosis (Zhang *et al*., 2022).

Most of the patients, if not all, have positive ANA in their serum, ANA-negative SLE is extremely rare (Samanta *et al*., 2022) The hallmark of SLE is the presence of ANA, which is found in more than 95% of affected patients (Ameer *et al*., 2022b). ANA is positive in the majority of patients with SLE but is not specific for SLE (Pile and Kennedy, 2008). Like other immunological illnesses, SLE frequently exhibits ANA, which can be utilized for screening, diagnosis, and prognosis (Ameer *et al*., 2022b), in addition to serving as markers of disease, ANAs can have a direct role in the clinical manifestations of SLE, although this role depends on the specificity and amount of antibody present. Identification of any disease role requires the use of an assay that allows specific measurement of that antibody. The role of ANAs in disease, the properties of the target antigen are also important determinants of pathogenicity because, in some cases, the target antigens are the actual inducers of inflammation (Pisetsky and Lipsky, 2020).

2.8.1. 2. Anti-Double Strand Deoxyribonucleic Acid

Anti-double strand deoxyribonucleic acid (Anti-dsDNA) Antibodies to DNA another clinically important group of ANAs, they directed toward antigens on double-stranded (ds), be IgG or IgM in isotype, and be of high or low avidity. High-avidity IgG anti-dsDNA antibodies are the most clinically relevant of this group as they have a high specificity for SLE (Petty *et al*., 2015). (dsDNA) serve as diagnostic and prognostic markers (Bai *et al*., 2018).

Anti-dsDNA antibodies, which are regarded as one of the most distinctive forms, have a high specificity (96%) for SLE and are the criterion with the highest weight in the immunologic domain of the 2019 EULAR/ACR classification (Ameer *et al*., 2022b). DNA was the first of the nuclear antigens to be identified in 1957, high avidity immunoglobulin G (IgG) antibodies directed at double-stranded DNA

are highly specific for SLE (Pile and Kennedy, 2008). increased antibody concentrations of anti dsDNA may indicate disease activity (Pawlak-Buś and Leszczyński, 2022). Elevated levels of anti-dsDNA antibodies (Abs) in the blood is a characteristic sign of SLE but the titer of anti-dsDNA Abs can change during the disease depending on the disease activity (Andrianova *et al*., 2020).

Self-dsDNA involved in the pathogenesis of SLE. In SLE patients, the clearance of apoptosis, necrosis and neutrophil extracellular traps (NETs) are defective which is the major resource of self-dsDNA to induce autoantibody production with unrevealed mechanism. Anti-dsDNA antibodies are produced by autoreactive B cells with the help of autoreactive T cells. Immune complex of dsDNA and its autoantibody triggers the strong type I interferon (IFN-I) production through various intercellular DNA sensors in phagocytes and plasmacytoid dendritic cells (pDC). IFN-I is the driving force in SLE pathogenesis by participating in inflammatory reactions, tissue damage, DC maturation and activation of autoreactive T and B cells Figure (2-3) (Bai *et al*., 2018).

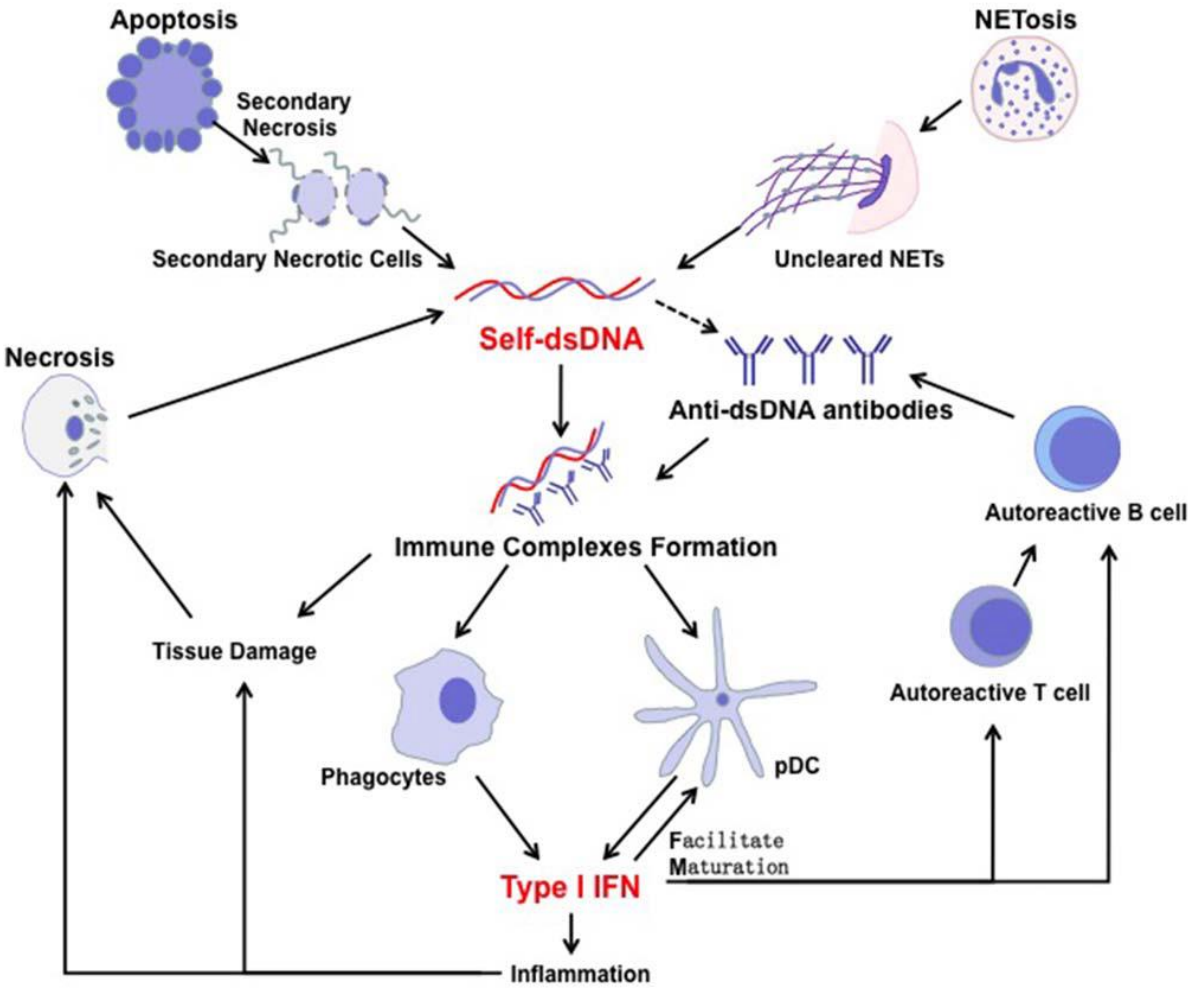


Figure (2-3): Production of self dsDNA that involved in pathogenesis of SLE (Bai *et al*., 2018)

2.8.2 Serum Complement

The complement system is a part of the immune system and consists of multiple complement components with biological functions such as defense against pathogens and immunomodulation. The complement system has three activation pathways: the classical pathway, the lectin pathway, and the alternative pathway. Increasing evidence indicates that the complement system plays a role in aging. Complement plays a role in inflammatory processes, metabolism, apoptosis، mitochondrial function, and Wingless Int-1 (Wnt) signaling pathways (Zheng *et al*., 2022a).

The most common laboratory findings of SLE low complement about (88%) of SLE patient show low complement (Timlin *et al*., 2018). decreased C3 and/or C4 complements, may indicate disease activity (Siddiqui *et al*., 2018) (Çelik *et al*., 2022). complement increases during the acute phase response, individual variability in complement gene copy number and expression, and variability in protein synthesis and catabolism. all these can influence plasma/serum complement levels, Because of these factors, low complement levels perform poorly as diagnostic markers for SLE (Weinstein *et al*., 2021).

SLE is recognized as a disease where autoantibodies develop and fix to self-antigen resulting in complement activation and leading to inflammation and tissue damage (Weinstein *et al*., 2021). The complement system, which consists of about 40 soluble and membrane bound proteins. The classical and the lectin pathways are activated through pattern recognition. Serine proteases bound to a pattern-recognition molecule are activated upon recognition of a fitting pattern and through several enzymatic reactions this lead to the activation of the classical and lectin pathway C3-convertase (C4b2a). The alternative pathway is in a state of constant activation, but is at the same time inhibited. There is a continuous hydrolyzes of C3 in the circulation, which can lead to complement activation via the alternative pathway of complement activation. Under normal condition, this process is inhibited both in the circulation and at the cell surface (Troldborg *et al*., 2018).

2.8.3. Hematological Finding in SLE:

Hematological abnormalities are common in SLE, both at the time of initial diagnosis and throughout the disease. The most frequent hematologic manifestations include Anemia, Leucopenia, Lymphopenia, Thrombocytopenia, Lymphadenopathy, and Splenomegaly (Santacruz *et al*., 2022).

No single measure can describe the condition in all SLE patients complex and standardized measures composed of clinical and laboratory variables have been developed to evaluate SLE disease activity, these measures use laboratory parameters like serum complement protein concentrations ،complete blood count (CBC), and anti–double-stranded (anti-dsDNA) antibody levels. However, there is still no reliable laboratory test that can independently measure SLE disease activity (Peirovy *et al*., 2020). CBC parameters are ideal biomarkers which are easily measured and sensitive to changes in disease activity. ESR was significantly high while RBC, WBC and platelet counts were significantly lower in SLE patients (Abira and Akhter, 2018). CBC and ESR changes in SLE patients are not known but literature review suggests that increased ESR may be due to chronic inflammatory response with increase in immunoglobulins (Abira and Akhter, 2018).

2.9. Osteoprotegerin (OPG):

OPG a glycoprotein mainly produced by osteoblasts, serves as a decoy receptor for RANKL. Osteoclast differentiation and subsequent bone resorption are inhibited as a result of OPG binding to RANK and blocking that protein's function. RANK, RANKL and the OPG system can form a tertiary complex, suggesting that OPG is not only a soluble decoy receptor for RANKL, but can also be considered as a direct effector of osteoclast functions (Ma *et al*., 2022). The molecular weight of OPG, which has 401 amino acids in it, is 60 kD. OPG can be found in the bloodstream as a monomer, homodimer, or in combination with its ligands, such as RANKL (Dutka *et al*., 2022). In addition to osteoblasts, there are cells that could express OPG. A recent study suggested that B cells are in charge of 64% of bone marrow OPG expression. OPG plays an anti-osteoclastogenesis role with binding to RANKL (Tobeiha *et al*., 2020). OPG is a member of the TNF receptor family. It has recently been demonstrated that OPG is produced by a variety of tissues, including the

cardiovascular system (Heart, Arteries, Veins), lung, kidney, immune tissues, and bone (Kiani *et al*., 2017) (Laymouna *et al*., 2021). has been identified as a regulator of bone resorption (Simonet *et al*., 1997).

It has also been demonstrated that granulocytes can generate IL-17 and OPG. When inflammation happens, IL-17 stimulates the production of multiple pro-inflammatory factors and increases the recruitment of granulocytes. Through IL-4 and IL-13, activated T lymphocytes also cause OPG expression in osteoblasts. The participation of OPG in the regulation of the activity of dendritic cells and the function of T and B lymphocytes (Dutka *et al*., 2022).

The expression and production of OPG is complex and is regulated by various cytokines and hormones (Hofbauer *et al*., 2000). Increased OPG levels have been seen in various autoimmune conditions including SLE, rheumatoid arthritis, Kawasaki's disease and Crohn's disease (Kiani *et al*., 2017).

OPG play a key role in the balance of osteoblasts and osteoclasts, which are very important in the regulation of bone metabolism in vivo and believed to be related to the reduction of bone mineral density in patients with SLE (Hao *et al*., 2022). As well (Kwok *et al*., 2009) concluded that OPG was higher significantly in SLE patients than in healthy individuals subjects, also (Kiani *et al*., 2017).

2.10. Osteocalcin (OC):

OC is a small non-collagenous protein synthesized by osteoblasts. Its synthesis is stimulated by 1,25-dihydroxyvitamin D. OC contains three glutamic acid residues, which are converted to gamma-carboxyglutamate by vitamin K-dependent post-translational carboxylation. The carboxylation process leads to a conformational change in protein which allows the binding of OC with hydroxyapatite and

mineralization in the bone matrix. there are also non-carboxylated or undercarboxylated forms of OC released into the circulation, but its significance in postmenopausal osteoporosis fracture prediction is less than intact OC (Hao *et al*., 2022). The production of OC is mainly by osteoblasts, odontoblasts, hypertrophic chondrocyte cells, and a small portion by adipocyte cells. (Sunarya *et al*., 2022) Osteocalcin is specifically expressed in osteoblasts and is the most abundant non-collagenous protein in bone (Komori, 2020).

OC have controlled several physiological processes in an endocrine, such as glucose homeostasis and exercise capacity, brain development, cognition and male fertility. Uncarboxylated Osteocalcin is released by osteoclasts and then binds its receptors to influence cognitive function, insulin synthesis in pancreatic b-cells, coinciding with exercise adaptation and testosterone secretion (Moser and van der Eerden, 2019). figure (2-4), OC play an important role in the regulation of glucose metabolism, as well as the muscle function of children (Zheng *et al*., 2022b). Bone is a dynamic tissue continuously under the influence of the counter-balancing actions of bone-forming osteoblasts and bone-resorbing osteoclasts. The effects of SLE on bone may thus act via pathways of bone formation, bone degradation or both (Baker-LePain *et al*., 2011).

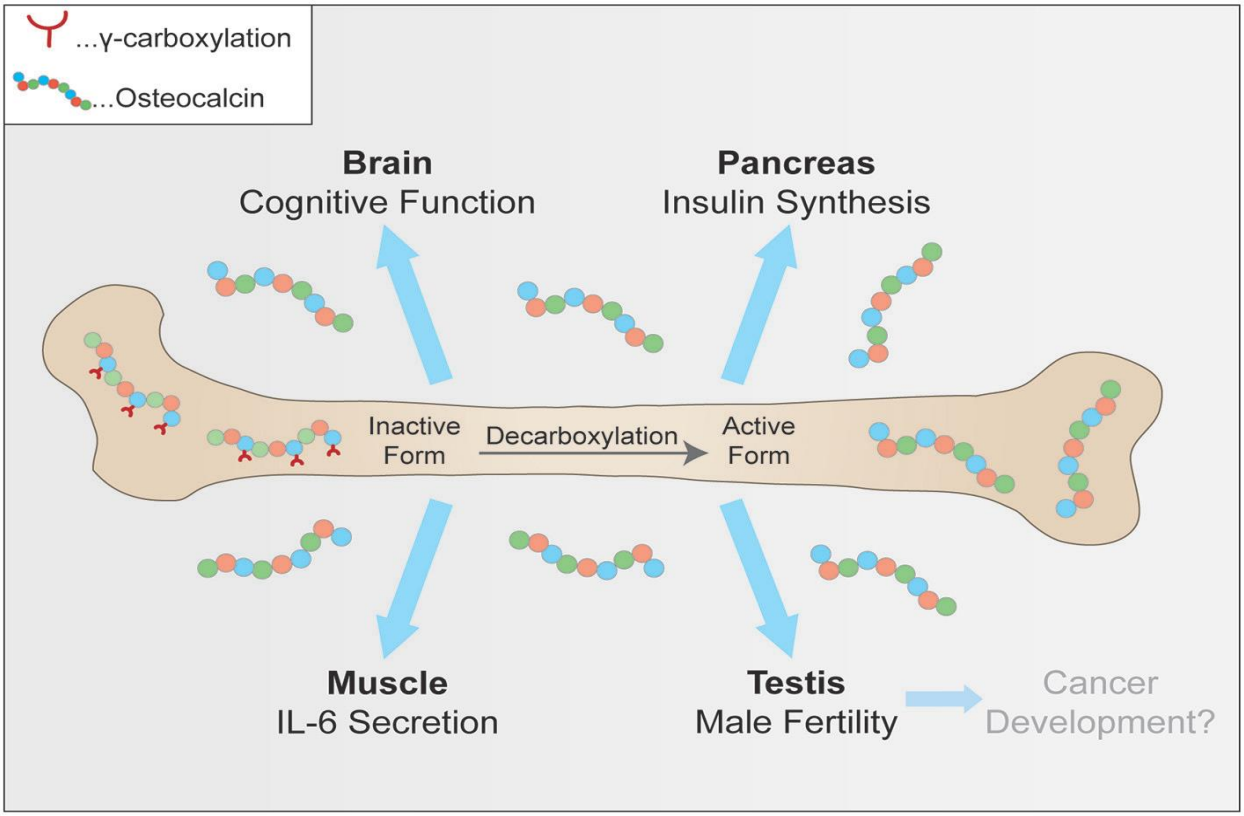


Figure (2-4) Physiological pathways controlled by Osteocalcin (Zheng *et al*., 2022b)

Three main types of bone cells are involved in the process of bone remodeling, namely osteoclasts, osteoblasts and osteocytes. Osteoblasts lay down and mineralize new bone matrix, while osteoclasts are responsible for bone resorption. Osteocytes mainly act as mechanosensors (Hlaing and Compston, 2014).

2.11. β-crosslaps (β-CTX):

The main component (~90%) of the protein matrix of bone. Beta-CTx is released into the bloodstream during bone resorption and almost entirely excreted by the kidney. Its serves as a specific marker for the degradation of mature type I collagen from bone (Azhari *et al*., 2020) (Arrieta *et al*., 2017).

Especially relevant fragments are the β-isomerized Carboxyterminal cross-linking telopeptides (β-CTx), produced osteoclastic hydrolysis of type I collagen

(Vasikaran *et al*., 2011)). These isomerized telopeptides are specific for the degradation of type I collagen in bone. Elevated serum concentration of isomerized C-terminal telopeptides has been reported for patients with increased bone resorption (Song, 2017) as Figure (2-5).

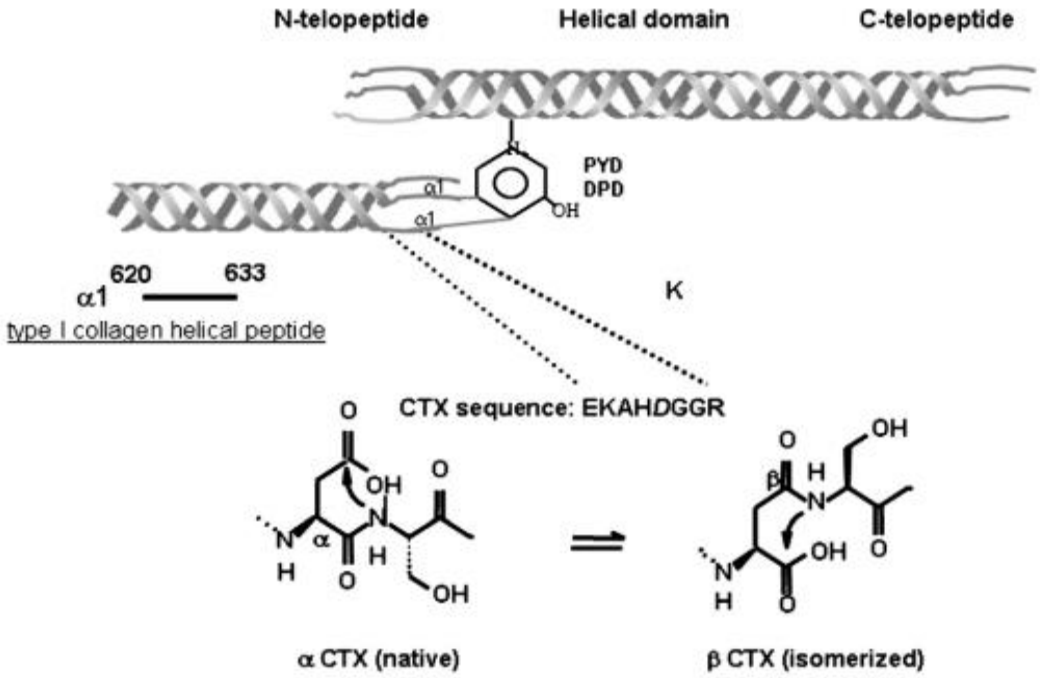


Figure (2-5): Schematic representation of the type I collagen molecule and the related degradation products (Garnero *et al*., 2009).

The β-CTx level is a biomarker of bone resorption, which depicted a decrease in bone quality in postmenopausal women (Azhari *et al*., 2020). Serum cross-linked C-telopeptide of type I collagen (CTX) is a marker of osteoclast activity and is used to assess the level of bone resorption (Lee and Suzuki, 2010). β -CTX act as bone resorption marker are elevated in SLE patients and positively associated with SLE disease activity in several studies (Bogaczewicz *et al*., 2015) (Zhu *et al*., 2021) (Guo *et al*., 2017).

2.12. Vitamin D:

Vitamin D, also known as calciferol, comprises a group of fat-soluble. The two major forms are vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) Vitamin D, in either the D2 or D3 form, is considered biologically inactive until it undergoes two enzymatic hydroxylation reactions. the first takes place in the liver, mediated by the 25-hydroxylase, the second reaction takes place in the kidney, mediated by 1α-hydroxylase, which converts 25OHD to the biologically active hormone, calcitriol (1,25-dihydroxyvitamin D) (Ross *et al*., 2011).

The best way to get vitamin D is through exposure to sunlight, The main source of vitamin D is cutaneous synthesis (Dominguez *et al*., 2021b). UV-B photons penetrate the epidermis and the absorbed energy causes the photolysis of 7-dehydrocholesterol, present in the plasma membrane of keratinocytes, into the previtamin D3 (Holick *et al*., 2007). The formed previtamin D3 is thermodynamically unstable and rapidly isomerizes to vitamin D3 (Holick *et al*., 1995). At 37 C, 80% of previtamin D3 is isomerized to vitamin D3 within 8 h. Generated vitamin D3 is released from the plasma membrane into the extracellular space, wherefrom it moves into the capillary and binds to plasma proteins. Skin synthesis can create 80–90% of the body's vitamin D under ideal conditions (Janoušek *et al*., 2022).

Synthesis of vitamin D upon UV-B radiation, the provitamins D ergosterol, 7 dehydrocholesterol, and 22,23-dihydroergosterolare respectively converted to pre-vitamins D2, D3, and D4, which are further thermally transformed into ergocalciferol (vitamin D2), cholecalciferol (vitamin D3), and 22,23-dihydroergocalciferol (vitamin D4). Only structures of pre-vitaminD3, and vitamin D3 are depicted for better lucidity (Janoušek *et al*., 2022). A quarter of skin surface (face, hands, and arms) would produce the equivalent of 1000 IU of vitamin D

(Dominguez *et al*., 2021a). VitD3 in serum SLE patients were significantly lower than those in normal control group (Hao *et al*., 2022).

Studies in SLE patients in different geographical regions demonstrate an increased frequency of vitamin D deficiency, a metabolic condition that induces bone loss. Moreover, low 25-hydroxyvitamin D (25[OH]D) serum levels were associated with low spine bone mineral density (BMD) in SLE patients (Bultink *et al*., 2012).

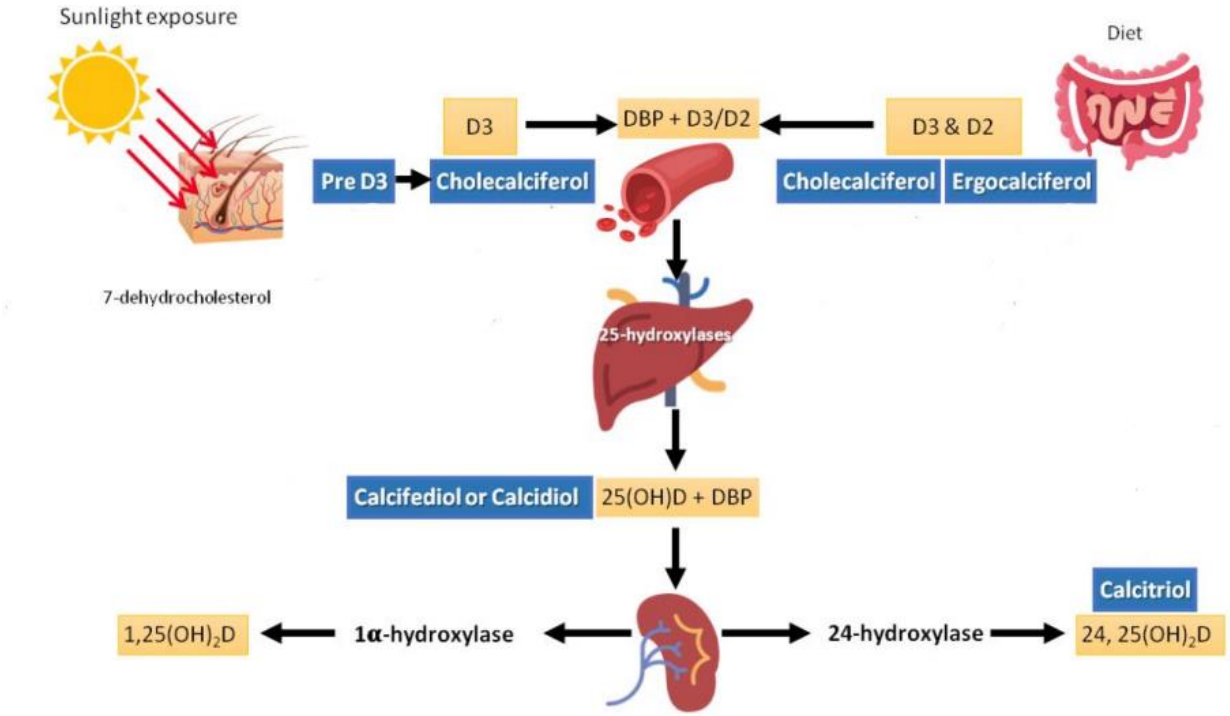


Figure (2-6) Cutaneous and dietary synthesis of Vit-D (Dominguez *et al*., 2021a).

2.13. Calcium:

Calcium as a nutrient is most commonly associated with the formation and metabolism of bone. Calcium metabolism is regulated in large part by the parathyroid hormone (PTH)–vitamin D endocrine system. Calcium is absorbed by active transport (transcellularly) and by passive diffusion (paracellularly) across the intestinal mucosa. Active transport of calcium is dependent on the action of calcitriol

and the intestinal vitamin D receptor (VDR). This transcellular mechanism is activated by calcitriol (Ross *et al*., 2011). Several studies have shown that calcium may help maintain optimum function of immune system and metabolism in SLE. Calcium homeostasis is maintained and regulated by 1,25(OH)2D3, which is the active form of vitamin D (Sha *et al*., 2020).

vitamin D has multiple effects on bone and calcium metabolism, Intestinal Ca absorption is regulated by 1,25 dihydroxy vitamin D (1,25(OH)2 D), a hormone that activates gene transcription following binding to the intestinal VDR (Fleet, 2022).

2.14. Blood Urea Nitrogen and Serum Creatinine:-

Blood urea nitrogen (BUN) is a clinical test to measure the amount of urea nitrogen in the blood. Urea is a product of protein metabolism in the liver. In fact, BUN is a non-protein nitrogenous (NPN) waste product of amino acids that is converted to urea by liver enzymes and later excreted by the renal system. However, the concentration of urea is dependent on protein intake BUN is influenced by various factors such as protein diet, variables in protein synthesis, the patient's hydration status and renal function status. (Rahbar *et al*., 2021).

Creatine is generally synthesized in the liver, pancreas, and kidneys. The produced creatine in then phosphorylated and converted to phosphocreatine in the skeletal muscle and brain. About 2% of creatine is converted to creatinine every day. Creatinine is a NPN by product of creatine breakdown the kidneys filter out most of the creatinine in urine. Elevated creatinine levels could suggest kidney dysfunction. Some temporary factors may cause a slightly higher serum creatinine (S.Cr) and BUN level. Persistently high level of S.Cr and BUN can result in severe kidney damage (Rahbar *et al*., 2021).

The 5-years survival rate for SLE patients with renal involvement is extremely low, even with treatment. Renal involvement affects a large number of patients and is responsible for significant morbidity and mortality in Western countries (Yang *et al*., 2012). The most prevalent test indicators of renal failure, regardless of the reason, are serum urea and creatinine. Additionally, lupus nephritis in SLE patients was independently associated with rising serum uric acid levels (Yang *et al*., 2011). Although the onset of lupus renal damage has been linked to several clinical SLE signs, according to several research (Yang *et al*., 2012).

Chapter three

Materials and Methods

Materials and Methods

3.1- Materials

3.1.1- Diagnostic Kits

All parameters were measured by standard kits as shown in table (3-1).

Table: (3-1): Diagnostic kits used in this study

No.	Test kits	Company-Origin
1	25-OH vitamin D3 ELISA Kit	MyBioSource (USA)
2	Osteoprotegerin ELISA kit	MyBioSource (USA)
3	Osteocalcin ELISA kit	MyBioSource (USA)
4	B-CrossLaps ELISA kit	MyBioSource (USA)
5	Complement C3 kit	Hipro (China)
6	Complement C4 kit	Hipro (China)
7	Anti-dsDNA kit	IDS / ISYS (UK)
8	ANA screen kit	IDS / ISYS (UK)
9	Urea kit	Human (Germany)
10	Cearіatinine kit	Human (Germany)
11	Ca^{+} kit	Human (Germany)

3.1.2- Instruments:

The instruments and other equipments that used in this study are shown in table (3-2).

Table: (3-2): Instruments and equipment's utilized in this study

No.	Apparatus and equipment	Company-Origin
1	ELISA reader	HumaReader HS (Germany)
2	Elisa plate	HumaReader HS (Germany)
3	IDS	IDS / ISYS(UK)
4	Selectora ProXL	ELITech Group France
5	Multichannel Micropipettes (0-250 µl)	HUAWEI (China)
6	Micropipettes 0.5-10 µl / 10-100 µl /100-1000 µl	Fisher Scientific (USA)
7	Vortex mixer	Karlkole (Germany)
8	Incubator	Memmert (Germany)
9	Centrifuge	Kokusan (Japan)
10	Deep freezer	GFL (Germany)
11	Eppendorff tube (1.5 ml)	Germany
12	ESR racks	AFCO(Jordan)
13	ESR tube	AFCO(Jordan)
14	EDTA K3 tube 2 ml Vacuum	AFCO(Jordan)
15	Disposable sterile blue, yellow and white tips	Germany
16	Disposable syringe 5- and 10-ml plastic	Medical jet (Syria)
17	Urine cups	Cybow (China)
18	Disposable test tube (10ml)	Afma.despo (Jordan)

3.2- Subjects:

This case control research was conducted on 131 SLE women over a period of 4 months from November 2022 till March 2023. SLE women included The current study were collected from the Baghdad hospital in medical city in (Baghdad governor). The practical part was occurred at Research Laboratories in International Center for Research and Development Located in the city of Kadhimiya and Teaching laboratories in the Medical City.

This research was approved by the Institutional Higher Scientific and Ethical Committee, and before participation all women were given an idea about the study and their written informed consent was taken. A special questioner was designed for all patients (appendix A).

All SLE women who are included in the study were subjected to physical examination, age, BMI, and laboratory investigations including urine examination, ESR, ANA, dsDNA, C3, C4, CBC, S.Ca, B.Urea and S.Cr.

Study Groups: One hundred and eighty-one women, these 181 women were divided into the following groups:

Healthy individuals group: 50 healthy women who do not have any signs or symptoms indicating that they are ill.

Patient group: - One hundred and thirty-one women with SLE were evaluated according to criteria used by (Bertsias *et al*., 2012), were used in diagnosed patients with SLE which include urinary casts, myositis, hematuria, proteinuria, pyuria, low complement and increased ds-DNA binding. Signs and symptoms such as arthritis, fever, new malar rash, alopecia and mucosal ulcers, subdivision were occurred (Bertsias *et al*., 2012), patient groups subdivided into three groups according to

disease severity which illustrated and classified according to Roma Helper program the disease severity which shown in table (3-3) and the three patient groups were:

- **Inactive SLE group**: This group is recognized if the patient has three degrees or less of disease activity, such as fever or low complement.
- **Mild and Moderate SLE group:** If the patient exceeds three degrees up to thirteen degrees, it is considered mild or moderate, as it is a combination of many signs and symptoms.
- **Severe SLE group:** In this group, the patient must exceed the thirteen degrees or more, as he becomes in a severe condition, as he has many signs and symptoms.

- Inclusion criteria: All women with lupus arthritis, whose age ranged from 15 to 65 years, and who have been examined clinically by a rheumatology doctor.
- Exclusion Criteria: Patients with Tumors, Patients under 10 years old, Persons taking nutritional supplements, other inflammatory disease such as RA, Osteoporosis, Myositis vasculitis, history of hypersensitive, Treatment Cyclophosamide, chronic condition such as asthma or crohn's disease, thyroid disease and pregnant.

Table (3-3) The Systemic Lupus Erythematosus Disease Activity Index (Bertsias *et al*., 2012)

D	S	Definition
Seizure	8	Recent onset. Exclude metabolic, infectious or drug-related causes
Psychosis	8	Altered ability to function in normal activity due to severe disturbance in the perception of reality. Includes hallucinations; incoherence; marked loose associations; impoverished thought content; marked illogical thinking; bizarre disorganised or catatonic behaviour. Exclude the presence of uraemia and off ending drugs
Organic brain syndrome	8	Altered mental function with impaired orientation or impaired memory or other intellectual function, with rapid onset and fl uctuating 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. rule out metabolic infectious and drug-related causes
Visual	8	Retinal changes from systemic lupus erythematosus cytoid bodies, retinal haemorrhages, serous exudate or haemorrhage in the choroid, optic neuritis (not due to hypertension, drugs or infection)
Cranial nerve	8	New onset of a sensory or motor neuropathy involving a cranial nerve
Lupus headache	8	Severe, persistent headache; may be migrainous
CVA	8	Cerebrovascular New syndrome. Exclude arteriosclerosis
Vasculitis	8	Ulceration, gangrene, tender finger nodules, periungal infarction, splinter hemorrhages. Vasculitis confirmed by biopsy or angiogram
Arthritis	4	More than two joints with pain and signs of inflammation
Myositis	4	Proximal muscle aching or weakness associated with elevated creatine phosphokinase /aldolase levels, electromyographic changes, or a biopsy showing Myositis
Urinary Casts	4	Heme, granular or erythrocyte

Haematuria	4	More than 5 erythrocytes per high power field. Exclude other causes
Proteinuria	4	More than 0.5 g of urinary protein excreted per 24 h. New onset or recent increase of more than 0.5 g per 24 h
Pyuria	4	More than 5 leucocytes per high power fi eld. Exclude infection
New malar Rash	4	New onset or recurrence of an inflammatory type of rash
Alopecia	4	New or recurrent. A patch of abnormal, diffuse hair loss
Mucosal ulcers	4	New onset or recurrence of oral or nasal ulceration
Pleurisy	4	Pleuritic chest pain with pleural rub or eff usion, or pleural thickening
Pericarditis	4	Pericardial pain with at least one of rub or effusion. Confirmation by ECG or echocardiography
Low complement	2	A decrease in CH50, C3 or C4 levels (to less than the lower limit of the laboratory determined normal range)
Increased DNA binding	2	More than 25% binding by Farr assay (to more than the upper limit of the laboratory determined normal range, eg, 25%)
Fever	1	More than 38 ^{o}C aft er the exclusion of infection
Thrombocytopenia	1	Fewer than 100 000 platelets
Leukopenia	1	Leukocyte count <3000/mm3 (not due to drugs)

3.2.1- Type of Samples: -

3.2.1.1-Blood Sampling:

Ten milliliters (ml) of venous blood were withdrawn from all SLE women using disposable syringes in the sitting position. The blood is discharged slowly into anticoagulant tubes and plain disposable test tubes. The blood was allowed to clot at 37˚C for 10-15 minutes, and then centrifuged at 1000 xg for approximately 10-15 minutes.

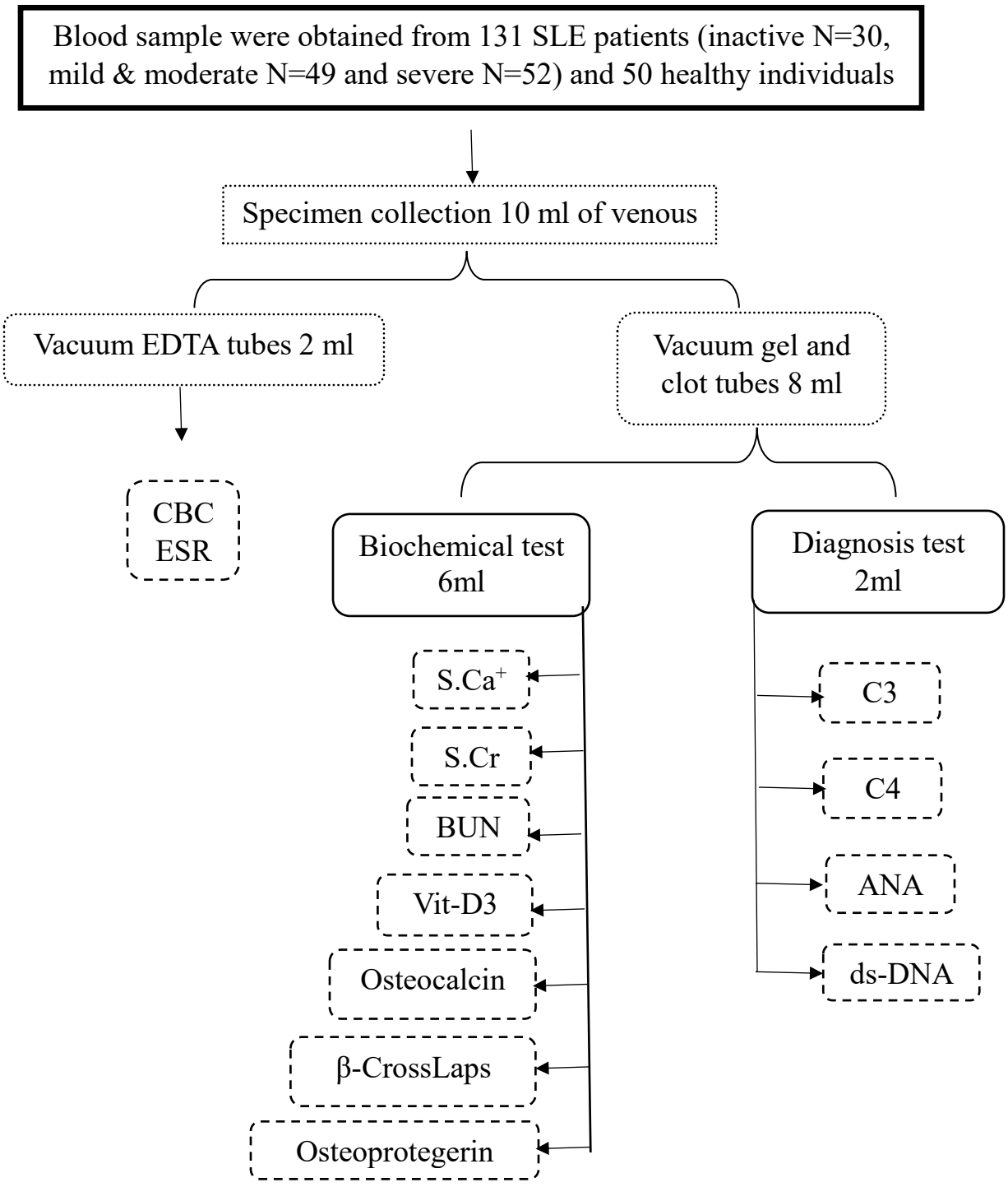
The blood was subdivided into three parts the first part was used in hematological test CBC and ESR were added in EDTA tube, and the second part (serum) used immediately for routine test that include serum ANA, dsDNA, C3, C4, S.Ca, B.Urea and S.Cr. And the third part stored in five eppendorf tubes at -80˚C until analysis of β-CTX, OPG, OC and VitD3 concentrations (Bertsias *et al*., 2012).

3.2.1.2-Urine Sampling:

Random urine sample was collected in sterile urine collection cup. SLE women were instructed to discard the first 20-25ml of urine and collect about 60 mL from the mid urine stream. These urine samples were used immediately for measurement of Urinary cast, hematuria Proteinuria and Pyuria (Bertsias *et al*., 2012).

3.3 General Design of the Study

General design of the study



3.4-Methods:

3.4.1- Determination of Human Serum Osteoprotegerin (OPG) Concentration:

Principle of the test: This assay employs the Double Antibody Sandwich ELISA technique. The pre-coated antibody was an anti-Human OPG monoclonal antibody, while the detection antibody was a biotinylated polyclonal antibody. Samples and biotinylated antibodies are added into ELISA plate wells and washed out with PBS or TBS after those additions to the wells. Then Avidin-peroxidase conjugates are added to the wells. TMB substrate was used for coloration after the enzyme conjugate washed out of the wells by PBS or TBS. TMB reacts to form a blue product from the peroxidase activity, and finally turns to yellow after addition of the stop solution (Color Reagent C). The color intensity and quantity of target analyte in the sample were positively correlated. Detection range of OPG: 0.4-0.6 ng/ml.

OPG Kit Components shown in Table (3-4).

Table (3-4): OPG Kit Components

No.	Name	96 Tests
1	Pre-coated plate	8×12
2	Human OPG Standards	2 vials
3	Biotinylated antibody (1:100)	1 vial
4	Enzyme conjugate (1:100)	1 vial
5	Enzyme diluent	1 vial
6	Antibody diluent	1 vial
7	Standard diluent	1 vial
8	Sample diluent	1 vial
9	Washing buffer (1:25)	1 vial
10	Color Reagent A	1 vial
11	Color Reagent B	1 vial
12	Color Reagent C	1 vial
13	Manual	1 set

Assay Procedure:

Sample and Test Preparation:

1. Serum: whole blood were collected then centrifuged at 1000 to 3000 rpm for 10 minutes, then supernatant taken tested right away.

2. Twenty minutes prior to the test, the ELISA kit from the refrigerator were removed and letted it come to room temperature.

3. Double-distilled water used to dilute the concentrated washing buffer (1:25).

4. To a lyophilized standard vial, 1.0 ml of Standard Diluent added and let stand for 30 minutes. After the standard has fully dissolved, it stirred briefly and labeled the tube with marker. The following concentration values used for the standard curve: 10, 5, 2.5, 1.25, 0.625, 0.312, and 0.156 ng/mL.

5. Standard sample dilution method: 7 clean tubes were selected, and their predicted concentrations (5, 2.5, 1.25, 0.625, 0.312, 0.156, and 0 ng/mL) were written on the labels. each tube with 300µl of standard diluent. 300µl of diluent pipetted from the reconstituted standard into the tube marked 5ng/mL, and then thoroughly mix. Further 300 µL of diluent pipetted out of the 5 ng/mL tube and added to the 2.5 ng/mL, then thoroughly mixed. Followed these instructions for the 0.156ng/mL standard as well. Standard The negative control is the diluent in the tube with 0ng/mL.

6. Biotinylated Antibody: the correct amount of biotinylated antibody solution removed for the number of wells that will be tested and diluted it 1:100 with antibody diluent. 30 minutes should be set aside for preparation.

7. Color Reagent: color reagent solution prepared 30min in advance by mixing color reagent A and color reagent B based on a 9:1 ratio.

8. Manual plate-washing: 350µl of wash buffer added to each well then waited 30 sec. Jolted the dish to remove of as much liquid, and if required, dabed with absorbent paper.

Assay Procedure Steps:

1. All kit components and samples bringed to room temperature (20-25 °C) before use.

2. Set aside Blank wells (skip the blank wells if measuring at dual wavelengths).

3. One hundred µL added to each well for standards or samples. The tape used to seal the wells/plate, then incubated at 37°C for 90 minutes.

4. ELISA plates three-washed

5. One hundred µl of biotinylated antibody produced was added to each well. Adhesive tape was placed on the reaction wells, then incubated at 37°C for 60 minutes.

6. The necessary amount of enzyme conjugate 30 minutes in advance was prepared.

7. The ELISA plate washed three times.

8. One hundred µl of prepared enzyme conjugate to each well were added other than the blank wells. The wells incubated at 37°C for 30 minutes after sealing them with the adhesive tape strip.

9. The ELISA plate was wash three times

10. One hundred µL of the prepared color reagent added to each well (including the blank well), Protected from light, incubated at 37 °C. The incubation was terminated when the coloration of the highest standards became darker and the color gradient occurred. Within 30 minutes, the chromogenic reaction should be under control.

11. Each well was geted 100 µl of color reagent c, as well as the blank well. Mixed thoroughly. within 10 minutes, the OD read at (450 nm), Figure (3.1).

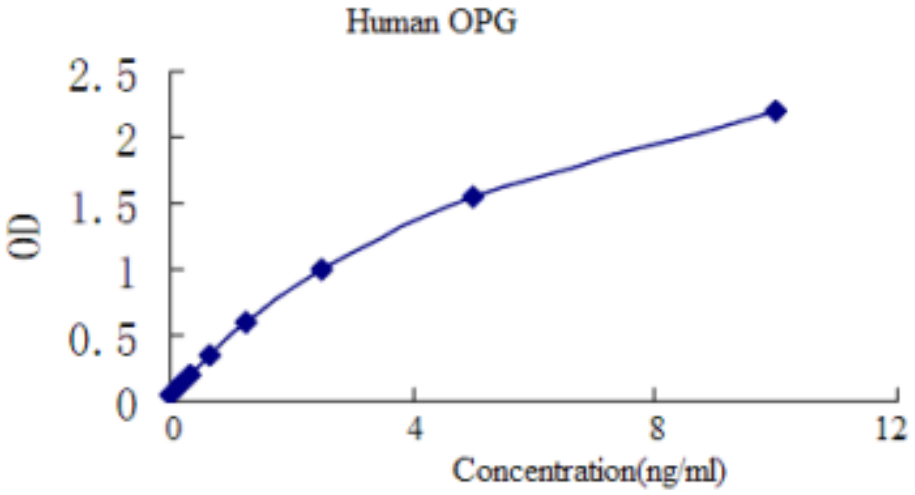


Figure (3-1): Standard curve of OPG concentration

3.4.2- Determination of Human Serum Beta-Crosslaps (βCTx) Concentration:

Principle of the test: Purified Human β-CTx antibody used to coat wells to make solid-phase antibody, then add β-CTx and βCTx antibody were added which has been labeled with (Horseradish Peroxidase) HRP to wells, then the reactants become antibody-antigen-antibody-enzyme complex, after washing completely, TMB substrate solution was added, TMB substrate becomes blue color under HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. β-CTx Kit Components shown in Table (3-5).

Table (3-5) βCTx Kit Components

No.	Name	96 Tests
1	Micro ELISA Strip plate wells	12×8 Strips
2	Standards×6 vials	0.5ml×6 vials
3	Sample Diluent	6.0ml
4	HRP-Conjugate Reagent	10.0ml
5	20×Wash Solution	25ml
6	Stop Solution	6.0ml
7	Chromogen Solution A	6.0ml
8	Chromogen Solution B	6.0ml
9	Closure Plate Membrane	2

Assay Procedure:

Sample and Test Preparation:

1.Serum - samples allowed to clot for 1 hour at room temperature before centrifugation for 10 min at approximately 3000 rpm. then the supernatants collected carefully.

2. Wash Solution (1×) - one volume of Wash Solution (20×) diluted with distilled water.

Assay Procedure Step:

1. Before beginning the assay method, all the reagents were prepared and let them letter naturally warm up to room temperature (18°C–25°C) for 30 minutes.

2. Standard wells, Sample wells, and Blank (Control) wells were set up, Standard 50µl was added to each Standard well, 50µl of sample were added to each Sample well, added Sample Diluent 50µl to each Blank/Control well.

3. One hundred µl of HRP-conjugate reagent added to each well, which covered with an adhesive strip, and incubated at 37 °C for 60 minutes.

4. Three times wash the Microtiter plate:

Manual Washing - incubation mixture removed by aspirating contents of the plate into a sink or proper waste container. a squirt bottle used, fill each well completely with Wash Solution, then the contents of the plate aspirated into a sink or proper waste container. repeated this process three times in total. After the last wash, the dish inverted and wiped it dry by slapping it against absorbent paper until there is no longer any moisture.

5. To each well, successively 50 µl of Chromogen Solution A and 50 µl of Chromogen Solution B were added. 15 minutes at 37°C of gentle mixing followed by light protection during incubation.

6. fifty µl of Stop Solution were added to each well. The color in the wells should change from blue to yellow.

7. The Optical Density (O.D.) read at 450 nm using a Micro ELISA Strip plate reader within 15 min.

Detection range of β-CTx: 810 - 970 ng/ml

3.4.3- Determination of Human Serum Osteocalcin (OC) Concentration:

Principle of the Test: A competitive enzyme immunoassay approach is used in the OC ELISA kit along with an anti-OC antibody and an OC-HRP conjugate. For one hour, the OC-HRP conjugate is incubated with the assay sample and buffer in a pre-coated plate. The wells are decanted and washed three times when the incubation period is over. The wells are then incubated with an HRP enzyme substrate. The

result of the enzyme-substrate reaction is a complex that is blue. The reaction is then stopped by adding a stop solution, which turns the solution yellow. In a microplate reader, the color density is measured spectrophotometrically at 450 nm. Due to competition for the anti-OC antibody binding site between OC from samples and OC-HRP conjugate, the density of the color is inversely proportional to the OC concentration. As more sites are taken up by OC from the sample, fewer sites are left that can bind OC-HRP conjugate because the number of sites is limited. A standard curve is plotted relating the density of the color (O.D.) to the concentration of standards. From this standard curve, the OC concentration in each sample is extrapolated. OC Kit Components in shown Table (3-6).

Table (3-6) OC Kit Components

No.	Materials	Specification	Quantity
1	Microtiter Plate	96 wells	Strip well
2	Enzyme Conjugate	6.0 mL	1 vial
3	Standard A	0 ng/mL	1 vial
4	Standard B	10 ng/mL	1 vial
5	Standard C	25 ng/mL	1 vial
6	Standard D	50 ng/mL	1 vial
7	Standard E	100 ng/mL	1 vial
8	Standard F	250 ng/mL	1 vial
9	Substrate A	6 mL	1 vial
10	Substrate B	6 mL	1 vial
11	Stop Solution	6 mL	1 vial
12	Wash Solution (100 x)	10 mL	1 vial
13	Balance Solution	3 mL	1 vial

Assay Procedure:

Sample and Test Preparation:

1. All kit components and samples bringed to room temperature (20-25 °C) before use.

2.Wash Solution - 10 mL of Wash Solution concentrate (100×) diluted with 990 mL of distilled water to prepare 1000 mL of Wash Solution (1×).

Assay Procedure Steps:

1.Added 100 uL of Standards or Samples to the appropriate well. Than added 100 uL of PBS (pH 7.0-7.2) in the blank control well.

2. fifty uL of Conjugate to added each well (NOT blank control well) and mixed well. the plate covered incubated for 1 hour at 37°C.

3. the microtiter plate washed using Manual Washing: Incubation mixture removed by aspirating contents of the plate into a sink. Each well completely fill in with 1× wash solution, and then aspirated contents of the plate into a sink. This procedure repeated three times for a total of three washes. After washing, the plate inverted, and blotted dry by hitting the plate onto absorbent paper or paper towels until no moisture appears. Complete removal of liquid at each step is essential to good performance.

4. fifty uL Substrate A and 50 uL Substrate B were added to each well including blank control well, subsequently then covered and incubated for 15-20 minutes at 37°C.

5. fifty uL of Stop Solution added to each well including blank control well and mixed well.

6. The Optical Density (O.D.) determined at 450 nm using a microplate reader immediately.

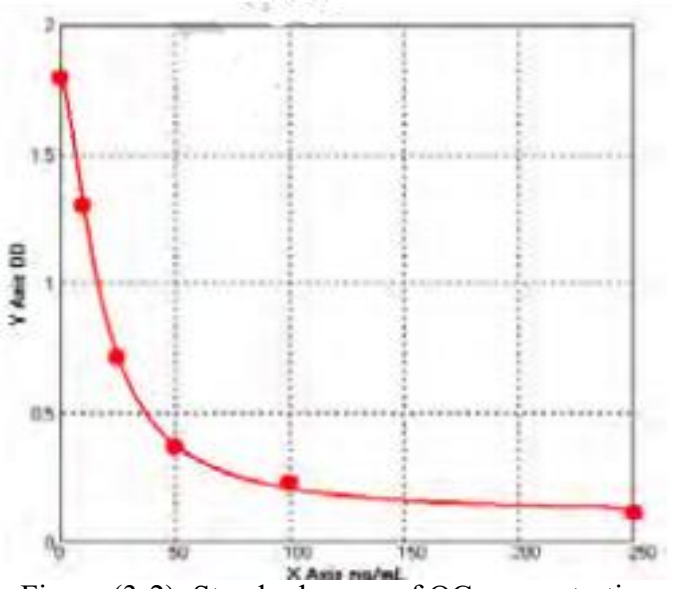


Figure (3-2): Standard curve of OC concentration

3.4.4- Determination of Human Serum 25-(OH) Vit-D3 Concentration:

Principle of the Test: This experiment use double-sandwich ELISA technique and the ELISA Kit provided is typical. The pre-coated antibody is human VD3 monoclonal antibody and the detecting antibody is polyclonal antibody with biotin labeled. Samples and biotin labeling antibody were added into ELISA plate wells and washed out with PBS or TBS. Then Avidin-peroxidase conjugates were added to ELISA wells in order; Use TMB substrate for coloring after reactant thoroughly washed out by PBS or TBS. TMB turns into blue in peroxidase catalytic and finally turns into yellow under the action of acid. The color depth and the testing factors in samples are positively correlated. Vit-D3 Kit composition shown in Table (3-7).

Table (3-7): Vit-D3 Kit composition

No.	Name	96 Tests
1	Antibody precoated plate	8×12
2	Human VD3 Standards	2 vials
3	Biotinylated antibody (1:100)	1 vial
4	Enzyme conjugate (1:100)	1 vial
5	Enzyme diluent	1 vial
6	Antibody diluent	1 vial
7	Standard diluent	1 vial
8	Sample diluent	1 vial
9	Washing buffer (1:25)	1 vial
10	Color Reagent A	1 vial
11	Color Reagent B	1 vial
12	Color Reagent C	1 vial

Assay Procedure:

Sample and Test Preparation:

1. Serum: entire blood was collected, and it was centrifuged for 10 minutes at 3000 rpm.

2. 20 minutes prior to the test, the ELISA Kit took out of the refrigerator and performed the test when it reached room temperature.

3. Distilled water used to dilute the concentrated washing solution (1:25).

4. Human VD3 standard sample: 1.0 ml of a standard diluent was added to a human VD3 lyophilized standard sample, and it was left there for 30 minutes. When the sample has completely dissolved, it mixed slightly and labeled the tube 1, then dilution taken as necessary. (the standard curve concentration values of 500, 250, 125, 62.5, 31.2, 15.6, and 7.8 ng/m were used).

5. Standard sample dilution method: The 7 clean tubes and name after that the numbers 2, 3, 4, 5, 6, and 7 prepared appropriately. Each tube filled with 300µl of the standard sample diluent. 300 µl of diluent pipetted from tube 1 to tube 2, then thoroughly combined. Further 300 µl of diluent pipetted from tube 2 to tube 3, then thoroughly mixed. The previous steps repeated up to tube 7. The tube 8 standard sample dilution serves as the adverse control.

6. Biotinylated human VD3 antibody liquid: created by diluting the concentrated biotinylated antibody with antibody diluent (1:100) according to the required dosage. 30 minutes should be set aside for preparation.

7. Enzyme-conjugate liquid: To make the enzyme-conjugate liquid, diluted the concentrated enzyme-conjugate (1:100) according to the required amount. 30 minutes should be set aside for preparation.

8. Color Reagent liquid: prepare the color reagent liquid 30 minutes in advance

with color reagents A and B in a 9:1 ratio.

9. Washing method: Manual plate-washing: 350µl lotion added to each well and keep it still for 30secs. Absorbent paper is used to clean each well after shaking it as thoroughly as possible.

Assay Procedure Steps:

1. The necessary strips from the bag removed to they have reach room temp.

2. blank wells were set aside (the blank wells may be skipped if a dual-wavelength reading plate is utilized).

3. The samples and the human Vit-D3 standard samples were added to wells (each well should hold 100 µl), 0ng/ml well filled with standard diluent. Adhesive tapes used to seal the reaction wells, and incubated for 90 minutes at 37°C.

4. The liquid biotinylated human VD3 antibody prepared 30 minutes beforehand.

5. The ELISA plate washed three times.

6. For each well 100 µl of the biotinylated human Vit-D3 antibody liquid were added. Using adhesive tapes to seal the reaction wells and incubated for 60 minutes at 37°C.

7. Enzyme-conjugate liquid prepared at least 30 minutes beforehand.

8. ELISA plate washed three times.

9. 100 µl of enzyme-conjugated liquid was added to each well except the blank wells. Using adhesive tapes to seal the reaction wells and incubated for 30 minutes at 37°C.

10. ELISA plate washed three times.

11. 100 µl of color Reagent liquid added to each well (along with a blank well), and incubated at 37 °C in a dark incubator. Hatching can be stopped when the color for a high concentration of the standard curve becomes darker and a color gradient form. Within 30 minutes, the chromogenic reaction were stopped.

12. 100 µl of color reagent C was added to each well in addition to the blank well. And mixed thoroughly. Within 10 minutes, OD at 450 nm was readed.

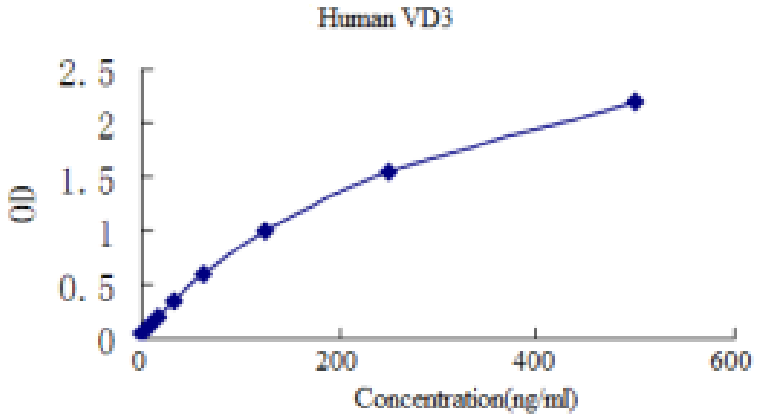


Figure (3-3): Standard curve of Vit-D3 concentration

3.4.5- Routine Biochemical Tests

Chemical analyzes such as S.Ca^{+}, B.Urea and S.Creatinine were measured by the Selectra device, this full automated device, where a tube containing serum was placed in the place designated for it, and then pressed on the Start button, as it took 10 minutes to obtain the results.

3.4.6- Hematological Tests

A 2 ml sample of venous blood in an EDTA tube was used to calculate the CBC and ESR.

3.4.6.1- Complete Blood Count

The blood components were measured by the CBC device, where the EDTA tube was placed in the place designated for it, and then the start button was pressed.

3.4.6.2- Erythrocytes Sedimentation Rate (ESR)

Add 2 ml of blood to a disposable ESR tube containing 0.5 ml of sodium citrate, thoroughly mixed the sample, and then manually pressed the sample up into a Westergren tube to the 200 mm mark. The tube was putted in an ESR rack at room temperature for 1 hour in a strictly vertical posture, during which time the distance between the upper limit of the red cell sediment and the lowest point of the surface meniscus was measured. This distance of erythrocyte fall was expressed in millimeters per hour.

3.4.7- Routine Immunological Tests:

3.4.7.1-Doubble Strand DNA (ds-DNA) and Antinuclear Antibody (ANA)

worked on IDS-ISYS machine that is powered by chemiluminescence immune assay to examine the of ANA and dsDNA. Where entered the kit for each examination in the place designated for it. After that, the samples were placed in the rack designated for them. And then give an order to the device to start. As the device was full automated, the scan takes about 25 minutes.

3.4.7.2- Complement (C3, C4)

Have worked on the Hipro device, which works with several techniques, including spectrophotometer, to measure the C3 and C4 complements. As the device is fully automated and the measurement time takes about 4 minutes.

The accessory of the Hipro device was the cuvette and sampler, Component of the integrated sampler, the reagent was pre-loaded, pipette-free. Appendix 2

Once the capillary tube was placed in the sample, about 3 microliters of it are sucked out, and then the sampler was placed in the cuvette, and then placed in the designated place in the device. Where the device pressed the injection valve, where the sample is mixed with the solution and incubated for 4 minutes, after which the device read the sample with the spectrophotometer feature.

3.4.8- Determination and Categorization of Body Mass Index (BMI):

Body mass index (BMI) was calculated according to the following equation:

$$\text{BMI} = (\text{weight in kilogram}) / (\text{height in meter})^2$$

According to World Health Organization, the BMI was separated into six categories (Jan and Weir, 2021) as shown in table (2-8):

Table (2-8): BMI classification(Jan and Weir, 2021)

Weight status	BMI range (kg/m^2)
Severely Underweight	<16.5
Underweight	<18.5
Normal weight	18.5 to 24.9
Overweight	25 to 29.9
Obese class I	30 to 34.9
Obese class II	35 to 39.9
Obese class III	>40

3.5- Statistical Analysis

By using Statistical Package for Social Science (SPSS 16 IBM, Armonk, USA), one-sample Kolmogorov-Smirnov test used to know how the values were distributed. If the values were distributed normally, then t- test is used and if the values are not distributed normally, so Mann-Whitney test should be applied. In this study the t-test was used for studying data. The results were expressed as mean ± standard deviation (SD), the differences in means of the variables between control and patient groups (inactive, mild and severe SLE groups) were analyzed by analysis of variance (one way ANOVA) test. Correlations between all of the studied variables were evaluated using Pearson's correlation coefficient (r) and linear regression analyses were used for the evaluation of data. *P*-value of < 0.05 was considered to be statistically significant. The receiver operating characteristic (ROC) curve was used to demonstrate the cut-off values, sensitivity, specificity and area under the curve of the studied research variables.

Chapter Four

Result and Discussion

4.1. Demographic and clinical characteristic of SLE patients and healthy individuals groups

The current study showed that there were insignificant difference ($p > 0.05$) in means of age in SLE patient groups (inactive, mild & moderate and severe) when compared with healthy individuals group. The results of demographic data were shown in Tables (4-1).

Table (4-1): Demographic characteristics of SLE patients and healthy individuals groups

Characteristics	Inactive SLE N (30)	Mild & Moderate SLE N (49)	Severe SLE N (52)	Healthy individuals Group N (50)
Age (Mean ±SD)	(31.83±12.12 **a N.S**	(33.75±11.51) **a.NS , d.NS**	(36.19±13.24) **a.NS, b.NS, c.NS**	(35.08±12.64)
BMI (Mean ±SD)	(28.40± 4.33) **a▲*****	(29.42±3.87) **a▲*** , d.NS**	(30.92±3.89) **a▲***, b▲* , c▲****	(25.21±1.66)

N: Number, SD: Standard deviation

a= ANOVA test between inactive, mild & moderate and severe groups versus healthy individuals: ▲*** =very higher significant increased (P <0.001), NS= Non-Significant.
b= ANOVA test between severe and mild & moderate groups: ▲* = significant increased (P <0.05).
c= ANOVA test between sever and inactive groups: ▲** = higher significant increase (P <0.01).
d= ANOVA test between mild and inactive groups: d=(N.S) =insignificant differences (P >0.05).

SLE is a chronic autoimmune disease that usually affects women, with mean age about (30.67 ± 8.27) (Kurniari *et al*., 2022), the characteristics data of The current study subjects showed that the mean age for total patients was 34.32±8.71 years old, and the entire sample was selected from females. Because systemic lupus erythematosus is more common in females; this is thought to be related to variants of the X chromosome and estrogen, which are known to have higher immune reactivity in females and contribute to triggering autoimmune diseases, including SLE (Sunarya *et al*., 2022). SLE is regarded as one of the top ten causes of death in

women under 65 years of age with a woman having about 9 times higher rate than a man for prevalence. Overall, the female immune system shows an increased reactivity by producing enhanced antibodies, increased activity of monocyte by more antigen presentation, a stronger type I interferon (IFN) response, and a higher rate of homograft rejection. The two main factors that play a key role in such reactivity are the estrogen hormone and genetics. SLE patients also showed increased alpha hydroxylation of estrogen, producing more estrogen metabolites which were responsible for activation of the T-cell and thus an increased differentiation of the B cell (Jabeen *et al*., 2021).

The comparison in levels of BMI between SLE groups (inactive, mild & moderate and severe) with healthy individuals group displayed in Table (4-1), revealed very high significant increase ($p < 0.001$) in means of BMI in severe, Mild & Moderate and inactive groups versus healthy individuals group. significant increase ($p < 0.05$) in means of BMI in severe groups versus Mild & Moderate group. significant increase ($p < 0.05$) in means of BMI in severe groups versus inactive group.; yet there was insignificant difference ($p > 0.05$) between Mild & Moderate and inactive groups.

This study is similar to (Chaiamnuay *et al*., 2007) the BMI scores were higher than normal, mean of BMI of these studies was 26.9 ±7.6 kg/m^2. In other study the results of the mean of BMI were 27±1.4 (Tedeschi *et al*., 2017).

according to World Health Organization (WHO) categories: normal BMI (18.5 kg/m^2 to<25 kg/m^2), overweight (25 kg/m2 to <30 kg/m^2) and obese (≥30 kg/m^2) elevated average BMI in SLE patients is a long-term measure of adiposity, associated with chronic exposure to inflammatory adipokines and circulating estrogens produced by adipose tissue, given the importance of systemic

inflammation and reproductive factors in the pathogenesis of SLE, there is strong biologic rationale for obesity increasing SLE risk (Tedeschi *et al*., 2017).

4.2. Major Manifestations of SLE

The result of clinical data was shown in table (4-2), were highest characteristic of arthritis, as its percentage in Severe group was (100%) and in the mild and moderate was (98%) (Appendix 3), the ds-DNA and complement in the severe group, where they were about (94.2%, 80.8%) respectively. While their percentage was half in mild and moderate about (59.1%, 57.1%) respectively. The ds-DNA and complement were the most common characteristic in inactive group was (53.3%, 33.3%) respectively. Mucosal ulcers in severe group were more than (85%), while in the mild and moderate was (46%). The percentage of rash characteristic was somewhat similar to the percentage of mucosal ulcers in the severe group, but in mild and moderate was about (18%). the percentage of fever in severe group was (46%), while in mild & moderate group was (16%), the percentage was the lowest in inactive group was (10%). urinary cast in severe group were (23%), while in the mild and moderate was (4%), and non-existent in Inactive group. the percentage of Pyuria in severe group was (23%), while non-existent in mild & moderate and inactive groups.

Vasculitis in Severe group was (17%), while non-existent in mild & moderate and inactive groups, was (13%) the percentage of hematuria and visual disturbance in severe group while non-existent in mild & moderate and inactive groups. Lupus headache in severe group was (9%) while in mild & moderate and inactive groups was (0%). the percentage of Proteinuria in severe group was (7%) and in mild & moderate group while non-existent in inactive group was (2%). Pleurisy in severe group was (1.9%), while non-existent in mild and moderate group, but in Inactive group was (6.7%).

In this study, the main feature of the patients was arthritis but this study also showed the prevalence of some manifestations such as oral ulcer, rash and Low complement was high compared to other criteria. The most frequent clinical indicator used to diagnose SLE is arthritis, in Dubai which affect about 88% of cases, in Saudi Arabia about (68%) and in Iran about (65%) (Osio-Salido and Manapat-Reyes, 2010).

The commonest clinical manifestation was arthralgia and/or arthritis, which occurred in 81.1%, malar rash (53.1%) in (Jordan). Arthritis or arthralgia ranged from 47.8% (Oman) to 95% (Lebanon), Pulmonary manifestations ranged from 7.5% (Oman) to 27% (Tunisia), myositis is uncommon among Arabs with SLE were (2.7%) (Adwan, 2018).

Table (4-2): Clinical manifestation of lupus arthritis patients

Clinical manifestation	Inactive N (30)	Mild& Moderate N (49)	Severe N (52)
Arthritis	0 (0%)	48 (98.0%)	52 (100.0%)
Increase dsDNA	16 (53.3%)	29 (59.1%)	49 (94.2%)
Mucosal ulcers	2 (6.7%)	23 (46.9%)	45 (86.5%)
Rash	2 (6.7%)	9 (18.4%)	44 (84.6%)
Low complement	10 (33.3%)	28 (57.1%)	42 (80.8%)
Alopecia	1 (3.3%)	12 (24.5%)	35 (67.3%)
Fever	3 (10.0%)	8 (16.3%)	24 (46.2%)
Urinary Cast	0 (0%)	2 (4.1%)	12 (23.1%0)
Pyuria	0 (0%)	0 (0%)	12 (23.1%)
Vasculitis	0 (0%)	0 (0%)	9 (17.3%)
Hematuria	0 (0%)	0 (0%)	7 (13.5%)
Visual disturbance	0 (0%)	0 (0%)	7 (13.5%)
Lupus headache	0 (0%)	0 (0%)	5 (9.6%)
Proteinuria	0 (0%)	1 (2.0%)	4 (7.7%)
Pleurisy	2 (6.7%)	0 (0%)	1 (1.9%)

N: Number, %: percent

Musculoskeletal symptoms are signs of an active disease. Most lupus arthritis patients have periodic polyarthritis, which can range in severity from moderate to

severe and is characterized by tenderness and swelling of the soft tissues around the joints. There may be arthralgia without arthritis. Myopathy may potentially overlap with polymiositis or be a side effect of long-term steroid therapy (Carey *et al*., 2008).

The frequencies of the various clinical manifestations in comparison to two published cohorts (Euro-lupus and Grupo Latino Americano), indicate there were no significant difference in the frequency of photosensitivity, serositis, neuropsychiatric manifestations or thrombocytopenia in Latin Americans when compared to Euro-lupus cohort. Fever, malar rash, Raynaud's phenomenon, arthritis, thrombosis and myositis were less frequent than in the European cohort. Lymphadenopathy, discoid rash, oral ulcers, renal involvement, pulmonary and hemolytic anemia, on the other hand, were more common in Arabs than in Europeans. Hemolytic anemia particularly had a very high frequency 45.6%, 8% Europeans and 11.8% in Latin Americans (Adwan, 2018).

4.3. Immunological, Hematological and Biochemical Results

4.3.1. Anti- Nuclear Antibodies (ANA)

Mean values of ANA levels for patient (inactive, Mild & Moderate and severe) lupus arthritis and healthy individuals groups were1.51± 0.74, 1.73 ± 0.81, 2.21±1.18 and 0.154± 0.07 IU/ml, respectively, as shown in the figure (4-1).

The positive ANA in all lupus arthritis patient was observed in 97 (74.04%) when the index value is more than 1, while negative ANA present in 34 (25.95%) when index value was below 0.97.

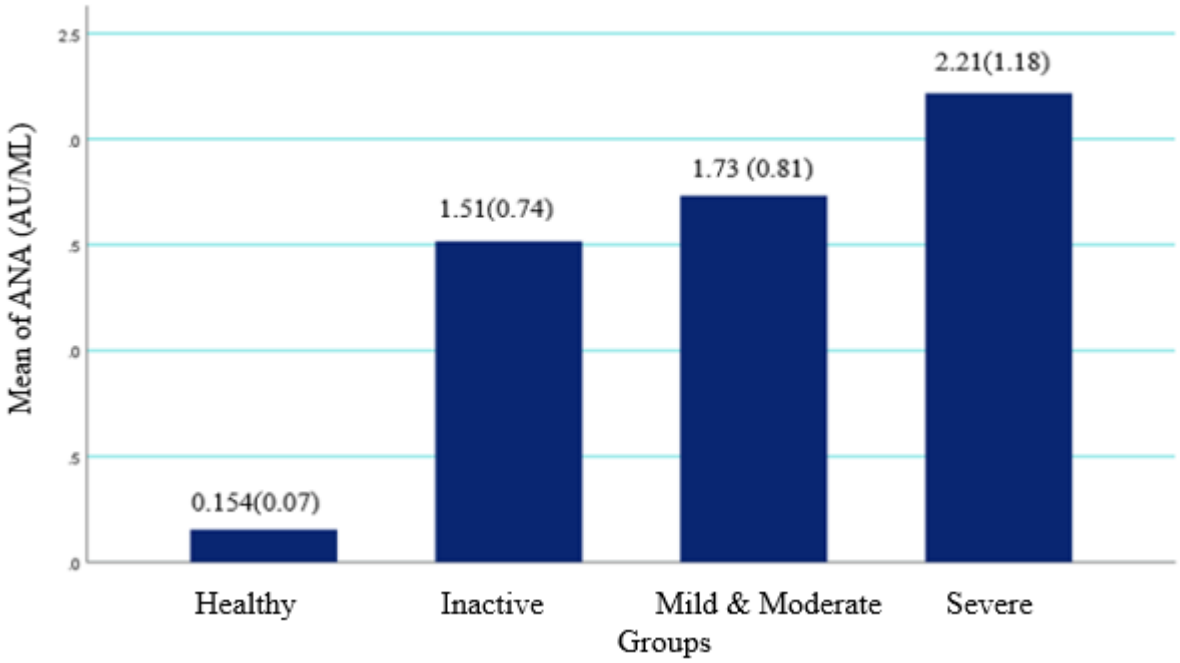


Figure (4-1): Anti-nuclear antibody in healthy individuals and patients (inactive, mild and moderate and severe) groups

The comparison in levels of ANA between lupus arthritis groups (inactive, Mild & Moderate and severe) with healthy individuals group displayed in Table (4-3), there were very higher significantly increased ($p < 0.001$) in means of ANA in inactive, mild & moderate and severe groups versus healthy individuals group. high significant increase ($p < 0.01$) in means of ANA in sever groups versus Mild & Moderate group; yet there were insignificant difference ($p > 0.05$) between Mild & Moderate and inactive groups.

The mean of ANA for all lupus arthritis patients (1.87±1.00) was statistically higher significantly at $p < 0.001$ when compared to the mean of healthy individuals (0.154± 0.07).

Table 4-3: ANA level in lupus arthritis groups (inactive, mild and moderate and severe) and healthy individuals group

Parameters	Groups			
	Inactive N = 30	Mild & Moderate N = 49	Severe N = 52	Healthy individuals N = 50
ANA **IU/ml**	1.51± 0.74 a▲***, Dns	1.73 ± 0.81 a▲***, c▲**	2.21±1.18 a▲***, b▲***	0.154± 0.07

a= ANOVA test between inactive, mild & moderate and severe groups versus healthy individuals: ▲*** =very higher significant increase (P <0.001).

b= ANOVA test between inactive and severe groups: ▲*** =very high significant increase (*P* <0.001).

c= ANOVA test between mild & moderate and severe groups: ▲** = high significant increase (*P* <0.01).

d= ANOVA test between inactive and mild & moderate groups: d=(N.S) =insignificant differences. (*P* >0.05).

Manson and Rahman showed over 90% of patients with SLE have positive anti-nuclear antibodies (ANA) (Manson and Rahman, 2006). Carey *et al* revealed positive ANA level in 88.2% of the patients. ANA is the test that should be used to diagnose for most patient because it is positive in over 85% of cases, typically when symptoms first appear. However, positive ANA results do not always denote SLE, though. almost all patients with SLE are ANA positive insignificant titers (Carey *et al*., 2008).

Ali, showed the hallmark of SLE is the presence of ANA which is found in more than 95% of affected patients. The likelihood of SLE is low in patients with negative

ANA who do not have the full constellation of symptoms joint pain and rash (Ali, 2018).

4.3.2. Anti-Double Strand Deoxyribonucleic Acid (anti-dsDNA)

The positive anti-dsDNA for all lupus arthritis patients was observed in 94 (71.75%) when the index value > 50, while negative anti-dsDNA present in 49 (28.24%) when index value < 50, as shown the figure (4-2).

The mean of anti-dsDNA for all lupus arthritis patients (53.51± 17.86) was statistically very high significant at $p < 0.001$ when compared to the mean of healthy individuals (10.54± 2.74).

- Inactive (n= 30) with the mean anti-dsDNA (43.16± 17.81).
- Mild and moderate (n= 49) with the mean anti-dsDNA (47.47 ± 16.27)
- Severe (n= 52) with the mean anti-dsDNA (65.177 $\pm$ 12.14)

The positive anti-dsDNA was observed (71.75%)، these results were accepted with the Egyptian authors, where the positive results for dsDNA were about 73% (Mohamed *et al*., 2020).

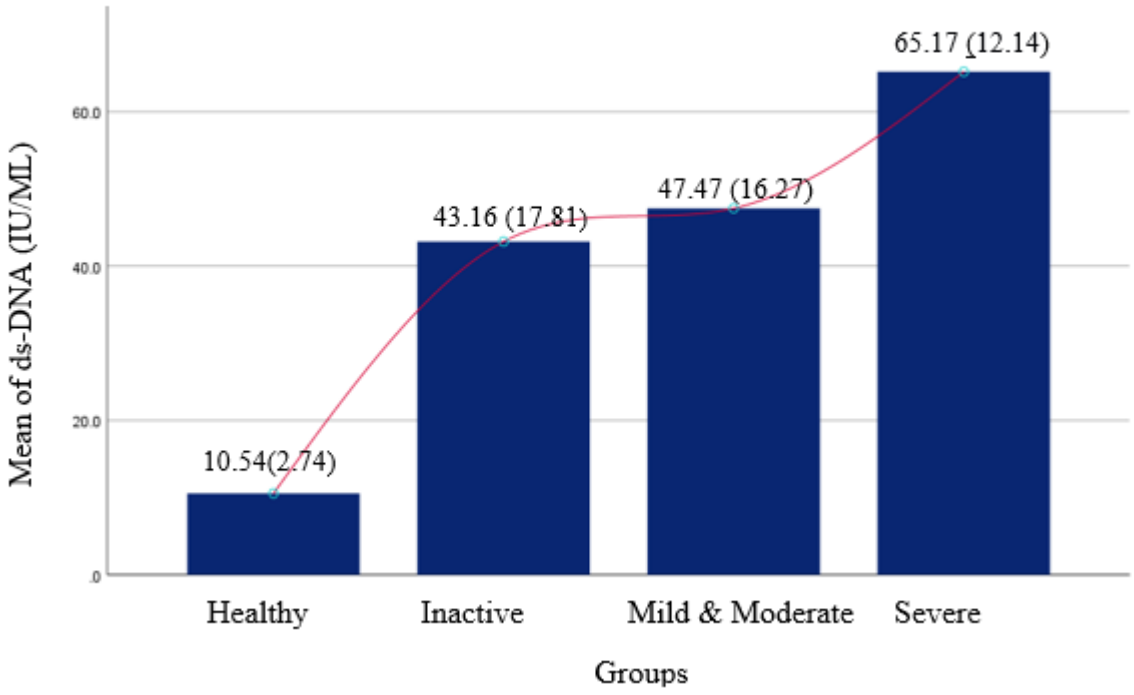


Figure (4-2): Anti-dsDNA in healthy individuals and patients (inactive, mild and moderate and severe) groups

The anti-dsDNA antibodies are useful for the estimation disease activity. The current study, comparison between groups, were very higher significantly differences, in comparing among any group (inactive, mild and moderate and severe) and healthy individuals group were very higher significantly increased Where the ($p < 0.001$). Also, when comparing between severe group and (inactive or mild and moderate) groups were very higher significantly increased ($p < 0.001$). in contrast when comparing the inactive and mild and moderate were insignificant difference ($p > 0.05$) between these groups.

Elevated levels of anti-dsDNA antibodies in the blood are a characteristic sign of SLE, but the titer of anti-dsDNA Abs can vary during the disease depending on the disease activity (Andrianova *et al*., 2020).

This study was conducted by a Chinese study the mean of serum ds-DNA antibody IU/mL (112.40 ± 10.82) (Guo *et al*., 2017).

Ali *et al* showed a high increase in the ds-DNA value, which was about (IU/ml) (267.6 ± 262.5) (Ali *et al*., 2019), while Zhu *et al* showed that approximately 65% of cases were abnormal in mean (30.7 ± 38.4) (IU/mL). While Bogaczewicz *et al* ds-DNA IU/mL was much higher than the previously mentioned results where they were (311.9 ± 397.0) (Bogaczewicz *et al*., 2015).

It is likely that extracellular DNA is one cause of an immune response against ds-DNA. There is a great deal of evidence supporting the idea that dead cells are one major source of this extracellular DNA (Su and Pisetsky, 2009). Apoptosis is the highly organized process of programmed cell death in which the cell degrades the nuclear DNA and signals for phagocytosis. In SLE patients and other autoimmune disorders this process is thought to be defective, causing either an increase in cell death and/or a decrease in the rate of dead cell clearance (Dieker *et al*., 2004).

4.3.3. The Result of Complements (C3, C4)

4.3.3.1. The Complements (C3)

C3 levels in sera from lupus arthritis patients were very higher significantly decreased as compared to healthy individuals (p <0.001). The mean concentration of C3 for lupus arthritis patients were (0.98± 0.40g/l) and ranged (0.30 – 1.84 g/l) as compared to the mean concentration of healthy individuals (1.59±0.28 g/l), lupus arthritis patients mean distributed as shown in the Table (4-4):

Table (4-4): The C3 level in lupus arthritis groups (inactive, Mild & Moderate and Sever) and healthy individuals group

Parameters	Groups			
	Inactive N = 30	Mild& Moderate N = 49	Severe N = 52	Healthy individuals N = 50
C3 g/l	**1**.17± **0**.42 a▼***	**1**.00± **0**.36 a▼***, d▼*	**0**.85 ± **0**.39 a▼***, b▼*** c▼*	1.59±0.28

a= ANOVA test between inactive, mild & moderate and severe groups versus healthy individuals: ▼***=very higher significantly decreased (P <0.001).

b= ANOVA test between severe and Inactive groups: ▼***=very higher significantly decreased (P <0.001).

c= ANOVA test between mild & moderate and severe groups: ▼*= significantly decreased (P <0.05).

d= ANOVA test between Mild & Moderate and inactive groups: ▼*= significantly decreased (P <0.05).

Comparing any lupus arthritis patient groups to healthy individuals group noted very high significantly decreased ($p < 0.001$), also, severe group revealed very high significant decreased comparing to inactive group with ($p < 0.001$), while when comparing the severe group with mild and moderate noted significant differences ($p < 0.05$). also, when compared between mild and moderate group and inactive group noted significant differences. ($p < 0.05$).

4.3.3.2. The Complements (C4)

The C4 levels in sera from lupus arthritis patients were very higher significantly decreased as compared to healthy individuals (p <0.001). The mean concentration of C 4for lupus arthritis patients were (0.18± 0.14 g/l) and ranged

(0.02– 0.81g/l g/l) as compared to the mean concentration of healthy individuals (0.35± 0.11g/l), lupus arthritis patients mean distributed as illustrated in table (4-5).

In fact, comparing the severe and mild and moderate groups to healthy individuals group noted very higher significantly decreased ($p < 0.001$), Wille as comparing the inactive group to healthy individuals noted significantly decreased ($p < 0.05$).

Severe group comparing to mild and moderate group noted higher significantly decreased ($p < 0.001$), When comparing the severe mild and moderate groups to inactive group noted very higher significantly decreased ($p < 0.001$).

Table (4-5): The C4 level in lupus arthritis groups (inactive, mild and moderate and severe) and healthy individuals group

Parameters	**Groups**			
	Inactive N = 30	Mild & Moderate N = 49	Severe N = 52	Healthy individuals N = 50
C 4g/l	**0**.29±**0**.19 a▼*	**0**.19±**0**.11 a▼***, c▼** d▼***	**0**.11±**0**.06 a▼***, b▼**, c▼**	0.35± 0.11

- a= ANOVA test between inactive, mild & moderate and severe groups versus Healthy individuals: ▼*** =very higher significantly decreased (P <0.001).
- b= ANOVA test between inactive and severe groups: ▼* = significantly decreased (P <0.05).
- c= ANOVA test between severe and mild & moderate groups: ▼** = higher significantly decreased (P <0.001).
- d= ANOVA test between mild & moderate and inactive groups: ▼*** =very higher significantly decreased (P <0.001).

Further studies (Li, Zhu and *et al*), revealed low complement C3 and C4 levels in serum (Zhu *et al*., 2021). Also, many studies confirm low C3 and C 4in SLE patients such as (Guo *et al*., 2017), (Ali *et al*., 2019), (Li *et al*., 2013).

The production of chemotactic factors, inflammatory modulators and antibody production, whereby antigens bind to C3b, which interacts with complement receptors and B cell receptors on B-type lymphocytes that activate, proliferate and produce antibodies (Ali *et al*., 2019). The complement pathway then assists in clearance of immune complexes and cell lysis via the membrane attack complex and removal of apoptotic cells and debris via C1q, C4b and C3b, which enhance the ingestion of dead cells by phagocytes (Sandhu and Quan, 2017).

There is loss of self-tolerance that results in autoantibody production in SLE patients with impaired clearance of apoptotic debris. C1q and other proteins typically assist in the removal of apoptotic material, which may explain why C1q deficiency has specifically been associated with impaired immune complex and apoptotic cell clearance (Sandhu and Quan, 2017).

4.3.4 Hematological test

4.3.4.1 Erythrocytes Sedimentation Rate

The result of the present study revealed that lupus arthritis disease was related to an elevated ESR and the mean was (48.96 ±17.06 mm/h) with range (19 -93 mm/h) when compared to healthy individuals (14.44±6.22 mm/h) range (4-30 mm/h).

Comparing any of the groups (inactive, mild & moderate and severe) to healthy individuals group noted very high significant increase in ESR levels with p <0.001, also, when comparing severe group to mild and moderate or inactive groups noted

very high significant at ($p < 0.001$), while mild and moderate compared to inactive groups noted insignificant differences ($p > 0.05$).

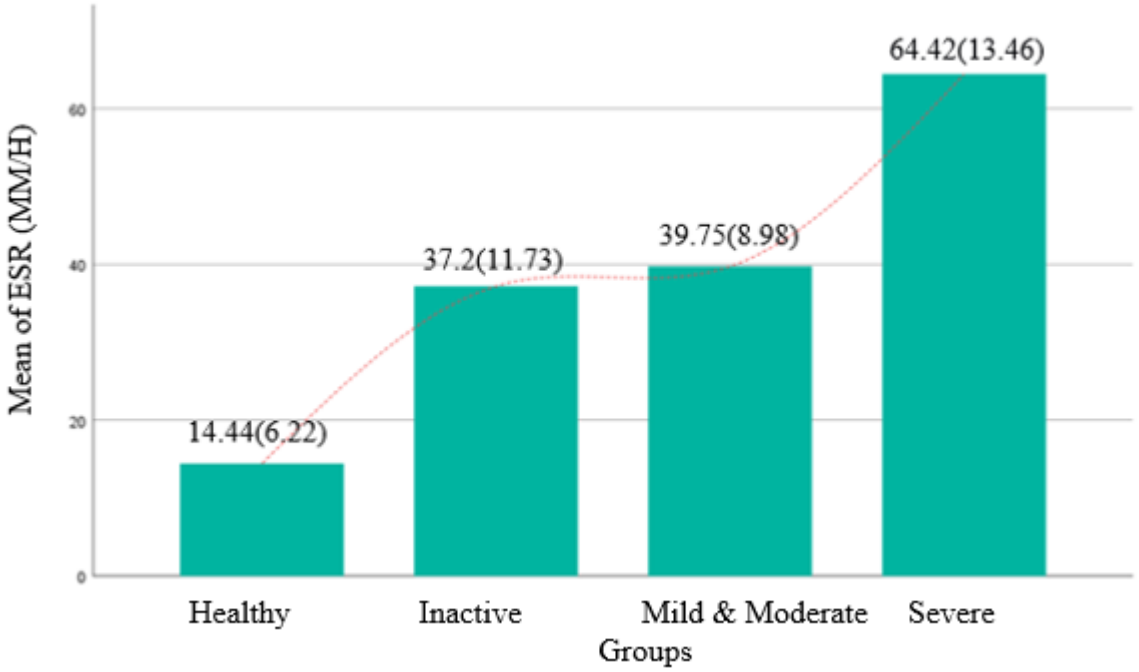


Figure (4-3): ESR in healthy individuals and patients (inactive, mild and moderate and severe) groups

These results were consistent with the results of Mohamed *et al* which showed elevated ESR level in severe group but the levels it was less the inactive group (Mohamed *et al*., 2020), as well as compatible with Guo *et al* that reported noticeable increase in ESR levels in SLE patients (Guo *et al*., 2017). There are as well many studies that have confirmed increased levels of ESR in patients with SLE (Ali *et al*., 2019) (Laymouna *et al*., 2021) (Abira and Akhter, 2018).

The changes in ESR for SLE patients is not known but literature review suggests that increased ESR may be due to chronic inflammatory response with polyclonal increase in immunoglobulins. (Elbagir *et al*., 2020) (Al Arfaj and Khalil, 2009). This increased ESR and might be consequence of increased inflammation process in SLE patients (Abira and Akhter, 2018).

4.3.4.2 Complete Blood Count (CBC):

The results of CBC of the study groups illustrated in Table (4-6):

Table 4-6: CBC level in lupus arthritis groups (inactive, mild & moderate and severe) and healthy individuals group

Parameters Mean ±SD	Inactive N (30)	Mild & Moderate N (49)	Severe N (52)	Healthy individuals N (50)
WBC	5.78(1.7)	5.38(1.5)	4.69(1.32)	6.04(1.21)
Hb	11.09(1.5)	10.89(1.45)	10.52(1.42)	12.38(0.7)
RBC	4.39(0.62)	4.1(0.45)	3.96(0.48)	4.91(0.52)
PLT	248.33(72.51)	236.73(71)	226.23(48.56)	288.9(30.06)

WBC values of lupus arthritis patients were compared with healthy individuals, there were very higher significantly decreased ($p < 0.001$) in means of WBC in severe group versus healthy individuals group, while mild and moderate group compared with healthy individuals group were significantly decreased ($p < 0.05$), but inactive group were insignificantly differenced ($p > 0.05$).

Significantly decreased ($p < 0.05$) in means of WBC in severe groups versus mild & moderate group, and higher significantly decreased ($p < 0.001$) versus inactive group; yet there was insignificantly differenced ($p > 0.05$) between mild & moderate and inactive groups.

The levels of Hb lupus arthritis groups (inactive, mild & moderate and severe) were compared with healthy individuals group revealed very higher significantly decreased ($p < 0.001$) in means of Hb in all groups versus healthy individuals group.

However, found no significant differences (p >0.05) in the comparison between severe and inactive groups.

The comparison in levels of RBC between lupus arthritis groups (inactive, mild & moderate and severe) implies very high significant decrease ($p < 0.001$) versus healthy individuals group, and when comparing severe group with inactive noted significant decrease ($p < 0.05$), but when compared the severe group to mild and moderate groups were no significant differences (p >0.05).

The levels of PLT of lupus arthritis groups (mild & moderate and severe) were compared with healthy individuals group showed very high significantly decreased ($p < 0.001$), while the inactive group noted high significantly decreased ($p < 0.05$) when compared to healthy individuals group. There are no significant differences (p >0.05) among groups.

In these results, clear differences noticed in CBC between the patients and healthy groups, and this is in line with many studies, including (Taha *et al*., 2022) (Abira and Akhter, 2018) (Abira *et al*., 2021) (Mohamed *et al*., 2020) (Shoeib *et al*., 2018).

Anemia, a common manifestation of SLE, may have many different causes. There is evidence to suggest that certain immunopathologic processes can also affect the erythropoiesis at several stages, before red blood cell maturation. The origin of primary anemia in lupus arthritis could be multifactorial, generated from a range of autoantibodies that can block erythropoiesis at several stages of red blood cell maturation (Tzioufas *et al*., 1997).

In SLE, antibodies directed against white cells are very common. A lower than normal lymphocyte count is found on the full blood count in lupus arthritis patients.

This is due to the presence of antibodies to lymphocytes which results in the destruction of the antibody-coated lymphocytes (Mahowald and Dalmasso, 1982).

(Yuri Gasparyan *et al*., 2011) revealed that high-grade inflammation accompanies a decrease of PLT in SLE, possibly due to the increased consumption of large platelets at the sites of rheumatoid inflammation.

4.2.5 Biochemical Tests

4.3.5.1 Blood Urea(B.Urea) and Serum Creatinine

The results of BUN levels in serum of lupus arthritis patients revealed very high significant increased ($p < 0.001$) compared to healthy individuals. The mean concentration of B.Urea for lupus arthritis patients were (31.56± 9.96) in comparison to the mean concentration of healthy individuals (20.38± 4.05) .

The results of S.Cr concentration in lupus arthritis patients serum revealed significant increase ($p < 0.05$) comparing to healthy individuals group. The mean concentration of S.Cr for lupus arthritis patients were (0.74± 0.30) and for healthy individuals were (0.60± 0.20) as showed in Table (4-7), and higher significantly increased when comparing inactive group ($p \leq 0.001$) to healthy individuals group. when comparing the severe group with mild and moderate group noted higher significantly increased ($p < 0.001$), and very high significant increase ($p < 0.001$) with inactive group, when comparing the mild and moderate group insignificant differences ($p >0.05$) to inactive group.

Table 4-7: B.U and S.Cr level in lupus arthritis groups (inactive, mild & moderate and severe) and healthy individuals group

Parameters Mean ±SD	Healthy individuals N (50)	Inactive N (30)	Mild & Moderate N (49)	Severe N (52)
B.U mg/dl	20.38(4.05)	26.83(6.37)	30.17(7.51)	35.63(12)
S.Cr mg/dl	0.60(0.2)	0.61(0.13)	0.72(0.26)	0.83 (0.37)

The severe group B.U levels when compared to healthy individuals group noted very higher significantly increased ($p < 0.001$), and when comparing the mild and moderate group with healthy individuals group noted significantly increased ($p < 0.05$), while comparing the inactive group noted insignificant differences ($p > 0.05$).

comparing creatinine levels in severe group showed significantly increased ($p < 0.05$) to mild and moderate group, and very high significant increased ($p < 0.001$) to inactive group, but when comparing between the mild & moderate and inactive noted insignificant differences ($p > 0.05$). It was shown that rising BUN and S.Cr levels were positively related to disease activity.

It is known that many studies have proven high blood urea and creatinine in lupus arthritis patients. The elevation may range between moderate and mild and severe. The current study, noticed a discrepancy between the levels, although all patients did not suffer from any symptoms regarding the kidneys, these results considered as predictive of kidney disease progression (Rani *et al*., 2021) (Häyry *et al*., 2022) (Yang *et al*., 2012) (Palazzo *et al*., 2022).

Urea is the major nitrogenous end product of the metabolic breakdown of protein in humans. It is dissolved in the blood and transported and excreted by the

kidney as a component of urine. The body continuously produces creatinine, which is a by-product of creatine phosphate found in muscle. The kidney is the only organ that primarily removes creatinine from the blood. It is filtered by the glomerulus and a small amount is also secreted into the glomerular filtrate by the proximal tubules. Blood creatinine rises as a result of reduced renal clearance (Kene *et al*., 2021). In SLE, the glomerular immune complexes are believed to be the primary mediators of renal disease. Recent studies make it apparent that autoantibodies of multiple specificities participate in the formation of immune complexes deposited in the kidneys. Renal infiltration by T cells, macrophages, and dendritic cells has a dominant role in the progression of lupus glomerulonephritis leading to renal failure (Bagavant and Fu, 2009).

4.3.5.2 Serum Ca^{+} ($S.Ca^{+}$)

The results of $S.Ca^{+}$ levels in lupus arthritis patient's serum revealed very high significantly decreased ($p < 0.001$) comparing to healthy individuals. The mean concentration of $S.Ca^{+}$ for lupus arthritis patients were (8.42± 0.47) in comparison to the mean concentration of healthy individuals (8.96± 0.58), these results for inactive, mild & moderate and severe were indicated in Table (4-8):

Table 4-8: S.Ca level in lupus arthritis groups (inactive, mild & moderate and severe) and healthy individuals group

Parameters Mean ± SD	Healthy individuals N (50)	Inactive N (30)	Mild & Moderate N (49)	Severe N (52)
S.Ca mg/dl	8.96(0.58)	8.87(0.4)	8.49(0.42)	8.0(0.29)

In comparing mild & moderate and severe group we noted very higher significant decreased ($p < 0.001$) to healthy individuals, but when comparing the inactive group to healthy individuals group noted insignificant differences ($p > 0.05$). When comparing severe group to any groups (inactive and mild & moderate) noted very high significantly decreased ($p < 0.001$), also mild and moderate group when compared to inactive group we noted very high significantly decreased ($p < 0.001$), but inactive group compared to healthy individuals were insignificant differences ($p > 0.05$).

Calcium homeostasis is maintained and regulated by 1,25(OH)2D3, which is the active form of vitamin D, low calcium may be due to Vit-D3 deficiency (Sha *et al*., 2020). Generally low S.Ca in SLE patients than healthy individuals, which is confirmed by many studies as (Laymouna *et al*., 2021) (Giannelou *et al*., 2020) (Sunarya *et al*., 2022) (Sha *et al*., 2020) (Guo *et al*., 2017) (Zhu *et al*., 2021), that explained in their studies lupus arthritis patients have low calcium levels, Ca levels is negatively correlated with lupus arthritis activity.

4.3.5.3. Vitamin D3:

The results of Vit-D3 were uneven between patients groups (Figure 4-4), where the severe group was characterized by a severe deficiency in the level of Vit-D3, where the mean ± SD of Vit-D3 was (10.1±1.66), and the results of the mild and moderate group were higher than the results of the severe group, which was (16.6±2.0), while the results of the inactive group were higher than mild & moderate and severe groups where they were (22.82±20). The highest results were the healthy group (33.46±4.84).

Comparing any patients groups (inactive, mild & moderate and severe) with healthy individuals group noted very high significantly decreased with ($p < 0.001$). also, when compare severe group to (inactive and mild & moderate) noted very high significantly decreased ($p < 0.001$), even when compare mild & moderate group to inactive group noted very high significantly decreased ($p < 0.001$).

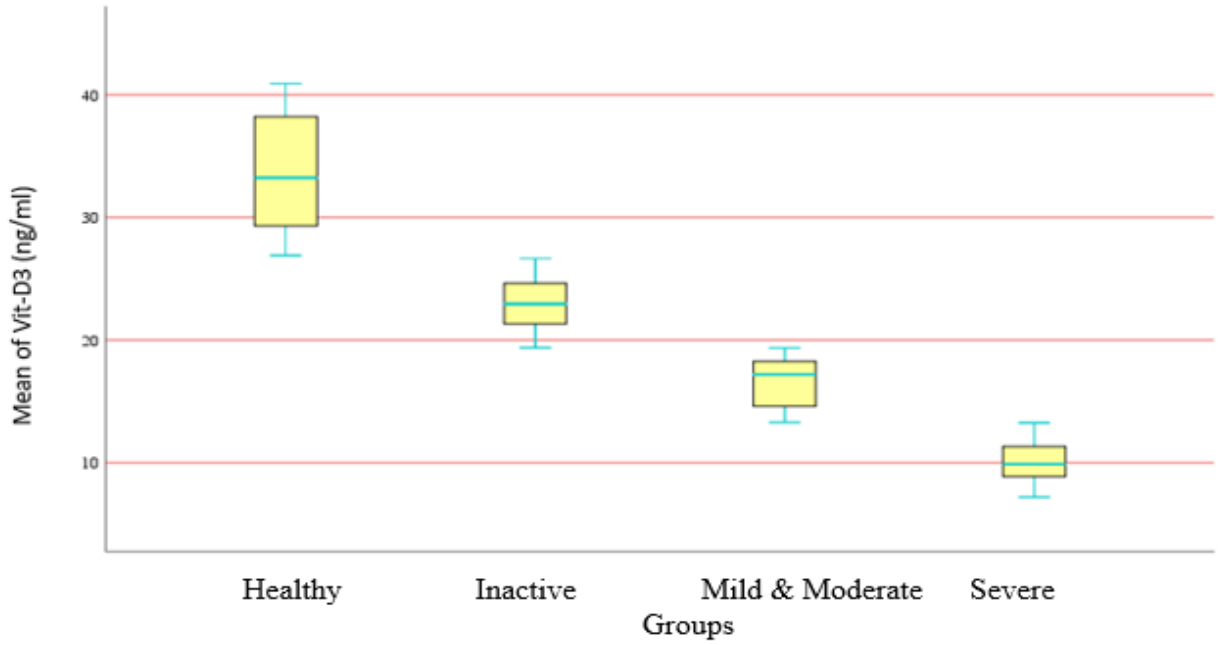


Figure (4-4): Vit-D3 in healthy individuals and patients (inactive, mild and moderate and severe) groups

Several studies reported a high prevalence of vitamin D deficiency in individuals with autoimmune diseases, SLE included (Souza *et al*., 2014). Tedeschi *et al* showed the patients with SLE have twice the fracture risk of individuals without SLE, numerous studies have demonstrated that lower vitamin D levels are associated with higher SLE disease activity (Tedeschi *et al*., 2019).

(Bogaczewicz *et al*., 2015) (Hao *et al*., 2022) (Giannelou *et al*., 2020) (Tedeschi *et al*., 2019) (Guo *et al*., 2017) (Zhu *et al*., 2021) they reveled during their studies were decreased Vit-D3 level in lupus arthritis patients.

Patients with lupus arthritis are at a clear risk of developing 25(OH) D deficiency because of photosensitivity and the frequently use of photoprotection (Ruiz-Irastorza *et al*., 2008). Although evidences showed the connotation between SLE and vitamin D through which SLE can lead to lower vitamin D levels, it is also important to consider the possibility that vitamin D deficiency may have a causative role in SLE etiology (Hassanalilou *et al*., 2018).

The correlation of the Vit-D values of severe group were positive significant correlation with RBC (r= 0.33, $p < 0.05$) as well showed weak positive significant correlation with B-CTX (r = 0.34, $p < 0.05$), Vit-D showed positive significant correlation with OPG (r = 0.4, $p < 0.01$). In contrast the Vit-D values showed weak negative significant correlation with C3 (r = -0.31, $p < 0.05$), while negative significant correlation with Hb (r = -0.38, $p < 0.01$). As for the mild and moderate group, there were no significant correlation Vit-D except with C3 showed weak negative significant correlation (r = -0.33, $p < 0.05$) as shown in the table (4-9).

Table (4-9): The correlation analysis using Pearson correlation of Vit-D3 in inactive mild & moderate and severe groups with C3, Hb, RBC, OPG and β-CTX parameters

Severe SLE		C3	Hb	RBC	OPG	β-CTX
VitD3	Pearson Correlation	-0.318*	-0.381**	0.339*	0.400**	0.346*
	Sig. (2-tailed)	0.022	0.005	0.014	0.003	0.012
Mild and Moderate SLE		C3				
VitD3	Pearson Correlation	-0.330*				
	Sig. (2-tailed)	0.021				

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

4.3.5.4. Osteoprotegerin

The result of OPG shown in the Figure (4-5): the inactive lupus arthritis patient, the OPG level were increased with mean (1.79±0.58), while patients mild and moderate patients have mean OPG level (2±0.51) were higher than whom with inactive group, the severe group was the highest level among the groups with mean (2.12±0.51). and the lowest values of the healthy individuals group were (0.51±0.07).

Comparing any groups of lupus arthritis patients to healthy individuals group noted very high significantly increased ($p < 0.001$), while when comparing severe group to mild and moderate noted insignificant differences ($p > 0.05$), but when compare severe or mild and moderate to inactive group noted significantly increased ($p < 0.05$).

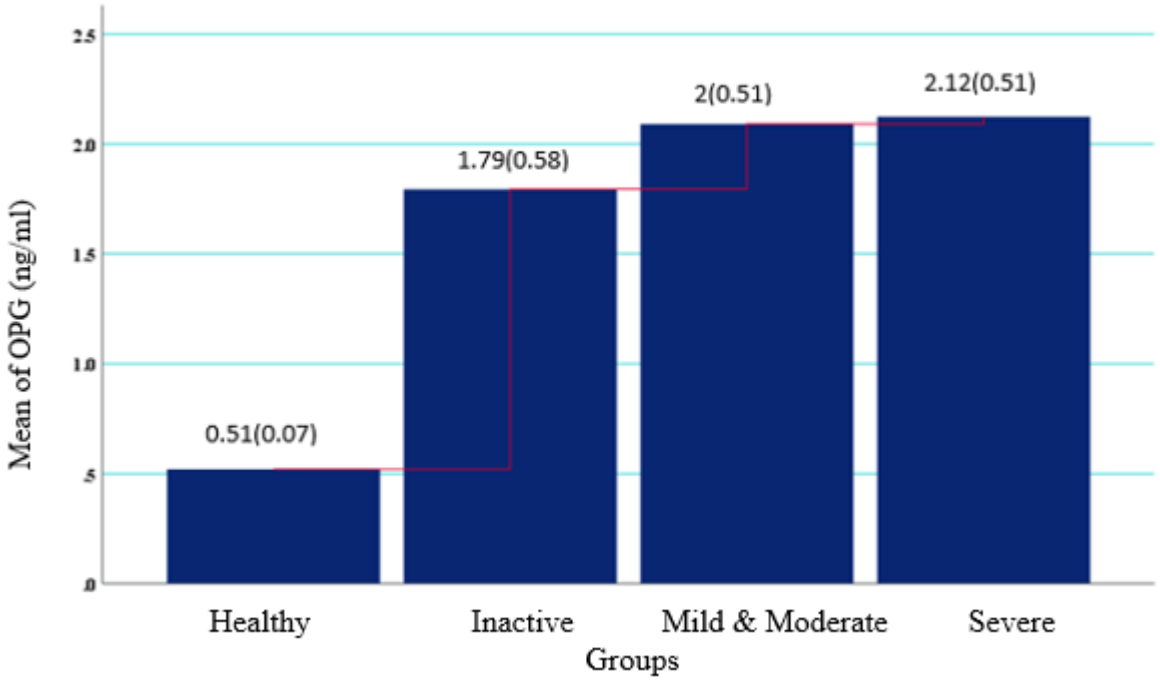


Figure (4-5): OPG in healthy individuals and patients (inactive, mild and moderate and severe) groups

The current study, found that OPG was statistically significantly higher increased in SLE patients than in healthy individuals. The current study agreed with (Park *et al*., 2014) who described a significantly higher level of OPG in SLE patients than healthy individuals. As well (Kwok *et al*., 2009) concluded that OPG was higher significantly in SLE patients than in healthy individuals subjects, also (Kiani *et al*., 2017) (Laymouna *et al*., 2021). The increase OPG may be due to an increase in the activity of B cells, which are responsible for 64% of OPG.

CD40 has either been indirectly or directly shown to be a contributing factor to the SLE disease, and CD40 levels are increased in SLE patients (Chen *et al*., 2015). During the disease, when the activity of B cells increases, the activity and production of CD40 also increase, CD40 has a role in the production of OPG. CD40

activation receptors lead to enhanced OPG production from B cells (Weitzmann, 2013).

lupus arthritis patients have increased leptin levels (Mohammed *et al*., 2018). Mechanism of action of leptin in bone is done by increasing OPG, which would inhibit osteoclastogenesis through the mediation RANK/ RANK ligand or OPG ligand /OPG. OPG functions as a soluble decoy receptor for RANKL and acts by competing with RANK, which is expressed on osteoclasts and dendritic cells for specifically binding to RANKL. The binding of RANKL to OPG would inhibit RANKL binding to RANK which then inhibits osteoclastogenesis (Legiran and Brandi, 2012).

In vitro studies have suggested that estrogen stimulates OPG expression. *In vitro* results give evidence that 17-estradiol enhances OPG secretion by osteoblastic cells (Kudlacek *et al*., 2003).

The expression of OPG is regulated by different hormones and cytokines, including 1,25(OH)2VitD3, the high expression of estrogen could increase the expressions of OPG (Hao *et al*., 2022).

The correlation of the OPG with (Vit-D3 and OC) for severe group, OPG showed positive significant correlation with Vit-D3 and OC (r= 0.40, $p < 0.05$), so in mild and moderate group the OPG appear positive significant correlation with S.Cr (r= 0.43, $p < 0.05$) while in same group the OPG appear strong negative significant correlation with B-CTX (r = -0.579, $p < 0.001$).

The correlation of the OPG with (ANA and ESR) of Inactive group OPG showed positive significant correlation with ANA and ESR (r= 0.40, $p < 0.05$) (r= 0.43, $p < 0.05$) respectively as shown in the table (4-10).

Table (4-10) The correlation analysis using Pearson correlation of OPG in inactive mild & moderate and severe groups with Vit-D3, OC, S.Cr, β-CTX, ANA and ESR parameters

Inactive SLE		ANA	ESR
OPG	Pearson Correlation	0.405*	0.430*
	Sig. (2-tailed)	0.026	0.018
Mild and Moderate SLE		**S.Cr**	**B-CTX**
OPG	Pearson Correlation	0.431**	-0.579**
	Sig. (2-tailed)	0.002	0.000
Severe SLE		**VitD3**	**OC**
OPG	Pearson Correlation	0.400**	0.404**
	Sig. (2-tailed)	0.003	0.003

**. Correlation significant at the 0.01 level (2-tailed).

*. Correlation significant at the 0.05 level (2-tailed).

The figure (4-6) explained positive correlation between OPG and Vit-D3 in severe group with $p < 0.0$ 1and $R^2 = 0.160$, as well as when correlation between OPG and OC with $p < 0.01$ and $R^2 = 0.164$. In contrast the figure (4-7) Explained negative correlation between OPG and B-CTX with $p < 0.00$ 1and $R^2 = 0.335$ in mild and moderate group.

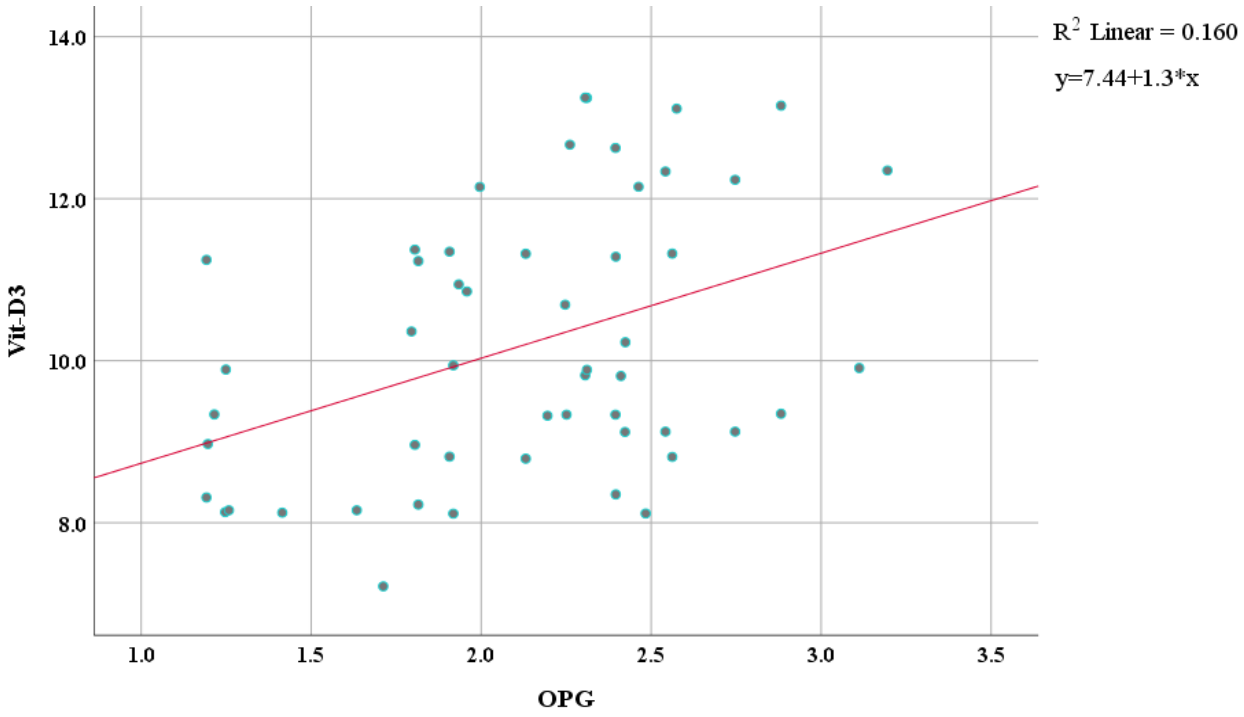


Figure 4-6: Correlation analysis in severe group between OPG and Vit-D3

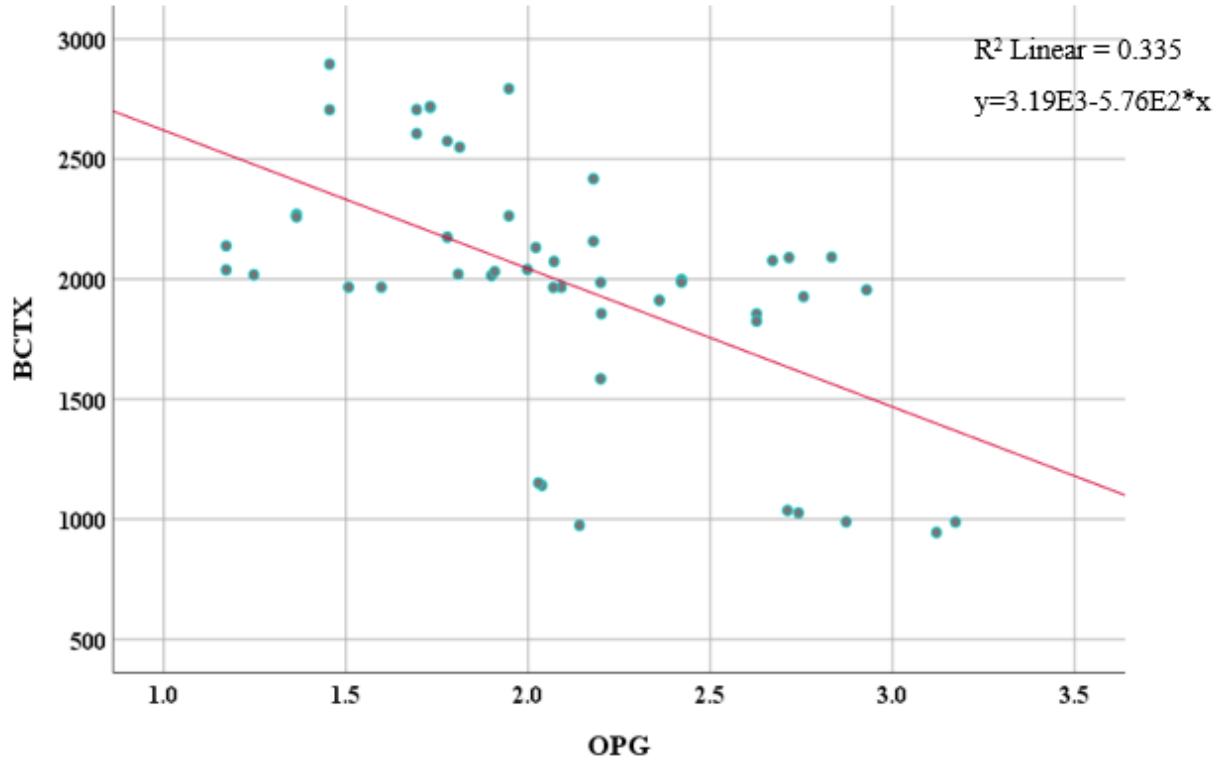


Figure 4-7: Correlation analysis in mild and moderate group between OPG and B-CTX

4.3.5.5. Osteocalcin:

The results of OC concentration in lupus arthritis patient and healthy individuals clarified in figure (4-8). The results of OC, noticed clear decreased in the patients groups compared to the healthy individuals. Comparing any patient groups (inactive, mild & moderate and severe) versus healthy individuals noticed very high significantly decreased ($p < 0.001$), while noticed slight differences between the patient groups, when compared the severe group to inactive group noticed significantly decreased ($p < 0.05$), but in compared severe group to mild and moderate noticed insignificant differences ($p > 0.05$), also, in compared the mild and moderate group to inactive group noted insignificant differences ($p > 0.05$).

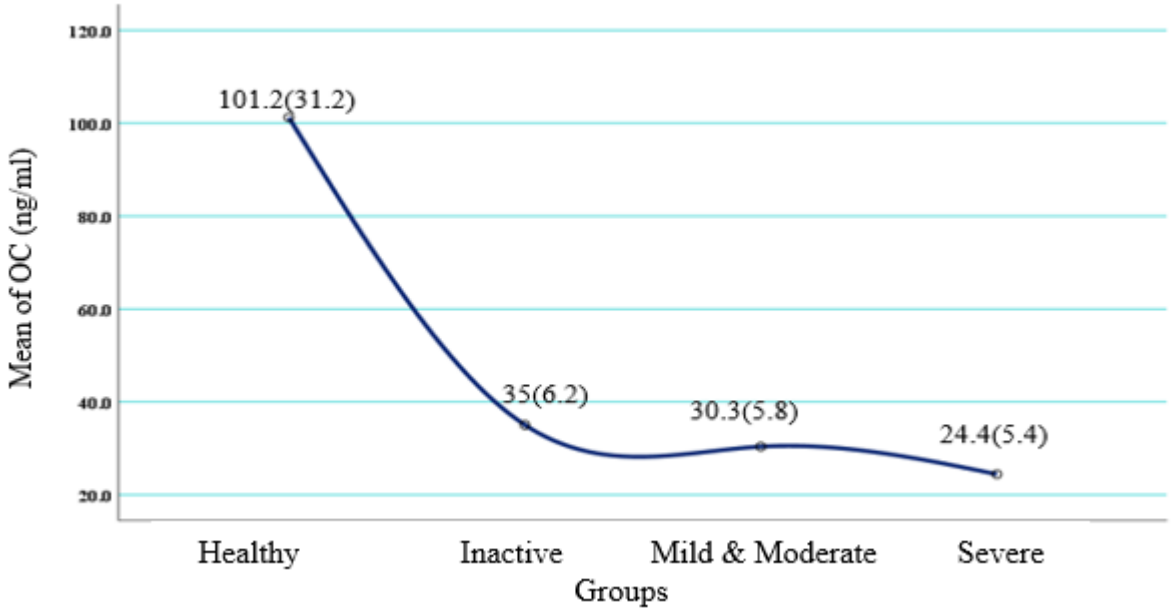


Figure (4-8): OC in healthy individuals and patients (inactive, mild and moderate and severe) groups

Other studeis revealed a decrease in OC level in lupus arthritis patients (Bogaczewicz *et al*., 2015) (Sunarya *et al*., 2022), as well many studies confirmed a decrease of OC in SLE patients (Baker-LePain *et al*., 2011) (Zhu *et al*., 2021) (Guo *et al*., 2017).

The current study agreement with (Baker-LePain *et al*) and (Guo *et al*) which state that SLE disease activity is negatively associated with osteocalcin as well (Baker-LePain *et al*., 2011) (Guo *et al*., 2017).

As mentioned earlier, OC is stimulated by 1,25 Dihydroxy cholecalciferol (125D), and therefore a deficiency of 1,25-dihydroxyvitamin D causes a decrease in the level of OC. Decrease in OC was observed, indicating an apparent uncoupling of bone remodeling in SLE patients that leads to bone loss and lower Bone mass density (Zhu *et al*., 2021).

Osteocalcin is released from the bone matrix and activated by the resorption activity of the osteoclasts; a process inhibited by OPG. Leptin is a potent inhibitor of osteocalcin through its effect on the sympathetic. (Ducy, 2011).

The correlation of the OC with (OPG) for severe group, the OC showed positive significant correlation with OPG ($r = 0.40$, $p < 0.01$), in mild and moderate group the OC appear positive significant correlation with Hb ($r = 0.47$, $p < 0.01$), and weak positive significant correlation with B-CTX ($r = 0.36$, $p < 0.05$), while the OC showed weak negative significant correlation with BMI ($r = - 0.289$, $p < 0.05$).

The correlation of the OC with (Age and C3) for inactive group, showed weak positive significant correlation with C3 and Age ($r = 0.40$, $p < 0.05$) ($r = 0.36$, $p < 0.05$) respectively shown in the table (4-11).

Table (4-11): The correlation analysis using Pearson correlation of OC in Sever Mild & Moderate and Inactive groups with OPG, BMI, Hb, β-CTX, C3 and Age parameters

Inactive SLE		C3	Age	
OC	Pearson Correlation	0.408*	0.368*	
	Sig.	0.025	0.045	
Mild and Moderate SLE		**BMI**	**Hb**	**β-CTX**
OC	Pearson Correlation	-0.289*	0.477**	0.363*
	Sig.	0.044	0.001	0.010
Severe SLE		**OPG**		
OC	Pearson Correlation	0.404**		
	Sig.	0.003		

**. Correlation significant at the 0.01 level (2-tailed).
*. Correlation significant at the 0.05 level (2-tailed).

The figure (4-6) explained correlation analysis in mild and moderate group positive correlation between OC and β-CTX with $p < 0.0$ 1and R^2 =0.132.

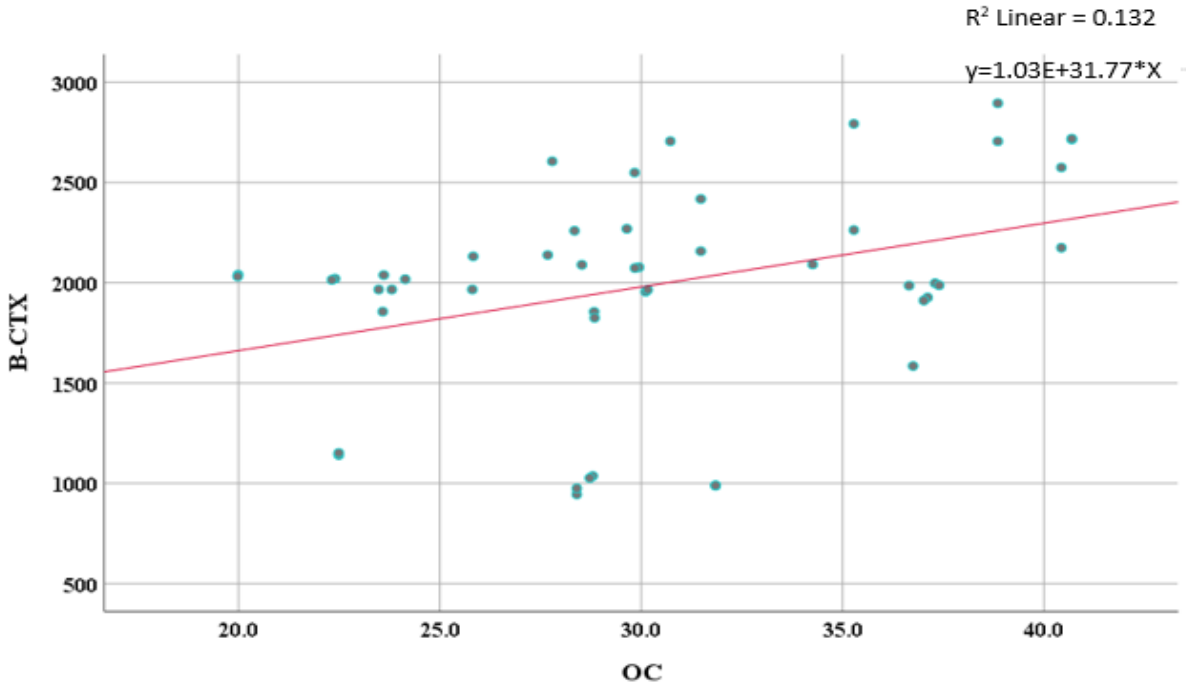


Figure 4-9: Correlation analysis in mild and moderate group between OC and B-CTX

4.3.5.6. Beta Crosslaps (β-CTX):

In this study, the levels of β-CTX were distinguished by the increase in concentrations for patients groups compared to the healthy subjects, as they were distributed as in the figures (4-10).

The result of the severe group were (2247±373), mild and moderate group (1991±515), and inactive group (1809±592), while healthy individuals was (883±52).

lupus arthritis patients groups (inactive, mild & moderate and severe) were compared with healthy individuals group noted very high significantly increased ($p < 0.001$), also when compared the severe group to inactive group noted very high significantly increased ($p < 0.001$), and when compared the severe group to mild and moderate group noted high significant increase ($p < 0.01$). In contrast when comparing the mild and moderate group to inactive group noted insignificant differences with ($p > 0.05$).

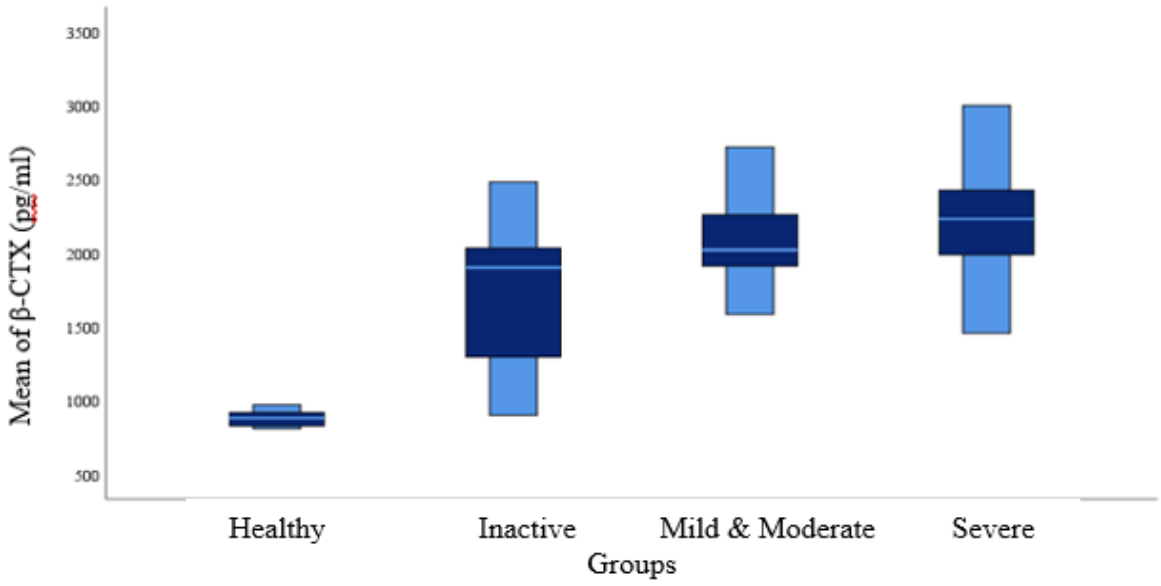


Figure (4-10): β-CTX in healthy individuals and patients (inactive, mild and moderate and severe) groups

β-CTX was positively associated with SLE disease activity, suggesting SLE disease activity, these results are consistent with Guo *et al* and Zhu *et al* Which also showed an increase in β-CTX compared to healthy individuals (Guo *et al*., 2017) (Zhu *et al*., 2021).

Bogaczewicz *et al* Suggested that β-CTX levels were elevated in SLE patients aged >45 in comparison to those at the age <45.That is, progress in age plays a role in the rise β-CTX (Bogaczewicz *et al*., 2015).

SLE disease is one of the main causes of its increase in bone resorption (Korczowska *et al*., 2003), β-CTX is released into the bloodstream during bone resorption and serves as a specific marker for the degradation of mature type I collagen. Elevated serum concentrations of β-CTx have been reported in patients with increased bone resorption (Christgau *et al*., 2000).

The correlation of the B-CTX of severe group showed positive significant correlation with WBC and Ca (r= 0.435, $p < 0.01$) (r= 0.40, $p < 0.01$) respectively. As mentioned earlier that B-CTX showed weak positive significant correlation with Vit-D3 (r = 0.34, $p < 0.05$) also the B-CTX appeared weak positive significant correlation with RBC (r = 0.30, $p < 0.05$). In contrast the B-CTX appear weak negative significant correlation with ANA (r = - 0.31, $p < 0.05$).

The correlation of the B-CTX of mild and moderate group showed weak positive significant correlation with OC, WBC and ESR (r= 0.36, $p < 0.05$), (r= 0.29, $p < 0.05$) and (r= 0.28, $p < 0.05$) respectively, in contrast the B-CTX appear negative significant correlation with S.Cr (r = - 0.39, $p < 0.01$). also, B-CTX showed strong negative significant correlation with OPG (r = - 0.579, $p < 0.001$).

The correlation of the B-CTX with (S.Cr and B.Urea) for Inactive group, B-CTX showed weak positive significant correlation with S.Cr (r= 0.36, $p < 0.05$), and

positive significant correlation with S.Cr (r= 0.36, $p < 0.01$), as shown in the table (4-12).

Table (4-12): The correlation analysis using Pearson correlation of OPG in inactive mild & moderate and severe groups with ANA, Ca, WBC, RBC, Vit-D3, ESR, S.Cr, OPG, OC and B.Urea parameters

Inactive SLE		S.Cr	B.Urea			
B-CTX	Pearson Correlation	0.368*	0.505**			
	Sig.	0.046	0.004			
Mild and Moderate SLE		**ESR**	**S.Cr**	**WBC**	**OPG**	**OC**
B-CTX	Pearson Correlation	0.282*	-0.392**	0.293*	-0.579**	0.363*
	Sig.	0.050	0.005	0.041	0.000	0.010
Severe SLE		**ANA**	**Ca**	**WBC**	**RBC**	**VitD3**
B-CTX	Pearson Correlation	-0.313*	0.405**	0.435**	0.300*	0.346*
	Sig.	0.024	0.003	0.001	0.031	0.012

**. Correlation significant at the 0.01 level (2-tailed).
*. Correlation significant at the 0.05 level (2-tailed).

The figure (4-11) explained positive correlation between B-CTX and Ca with $p < 0.0$ 1and R^2 =0.164, as well as when correlation between B-CTX and WBC with $p < 0.01$ and R^2 =0.164. The figure (4-12) Explain positive correlation between B-CTX and B.Urea with $p < 0.0$ 1and $R^2 = 0.255$.

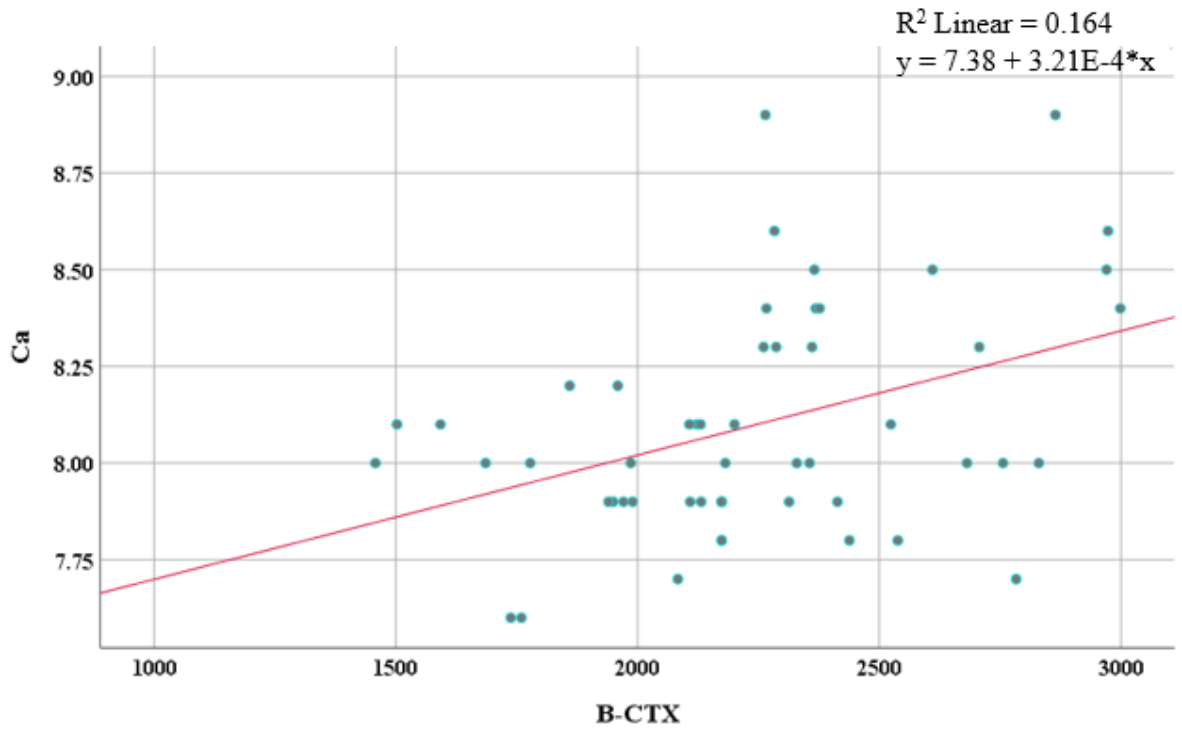


Figure 4-11: Correlation analysis in severe group between B-CTX and Ca

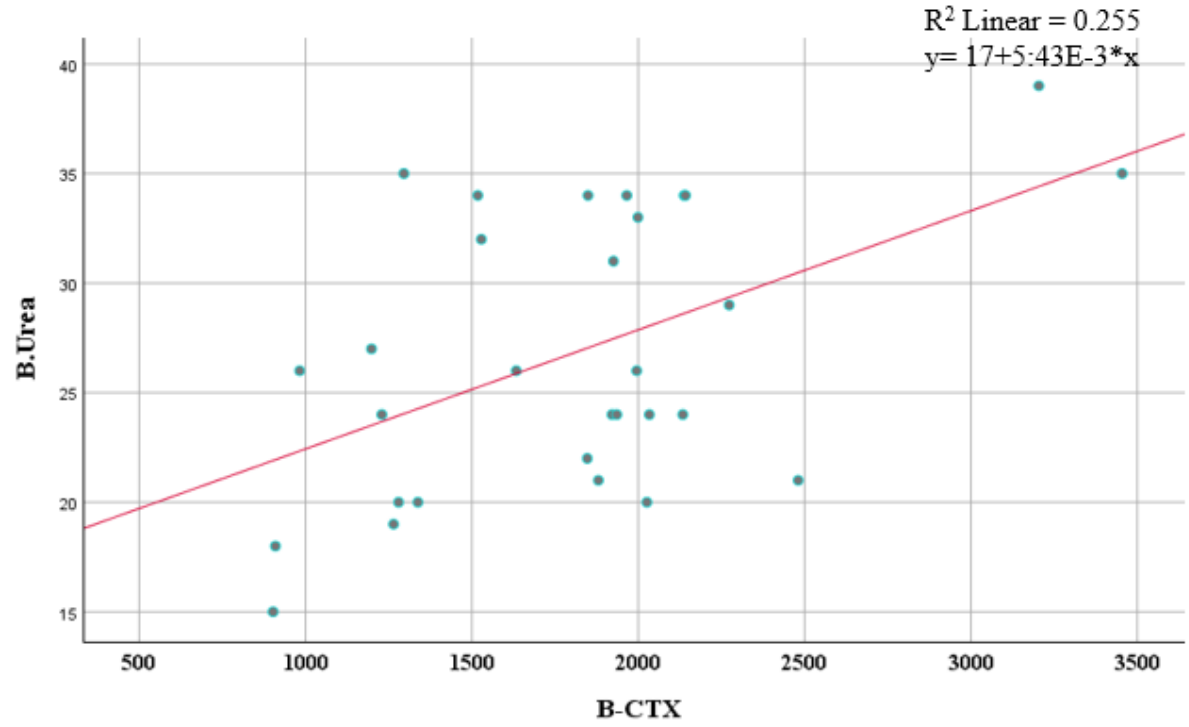


Figure 4-12: Correlation analysis in inactive group between B-CTX and B.Urea

4.4. Receiver operating characteristics for diagnostic markers of SLE.

Receiver operator characteristic curve was used for the analysis of significant differences of indices of lupus arthritis patient groups. Determination of the diagnostic performance is based on the area under the curve (AUC) as follows: AUC = 0.9–1.0, excellent; AUC = 0.8–0.9, good; AUC = 0.7–0.8, fair; AUC= 0.6–0.7, poor; and AUC <0.6, not useful (Anjum *et al*., 2020) (Rahman *et al*., 2021).

β-CTX showed excellent diagnostic performances according to ROC analysis, as shown in the figure (4-13).

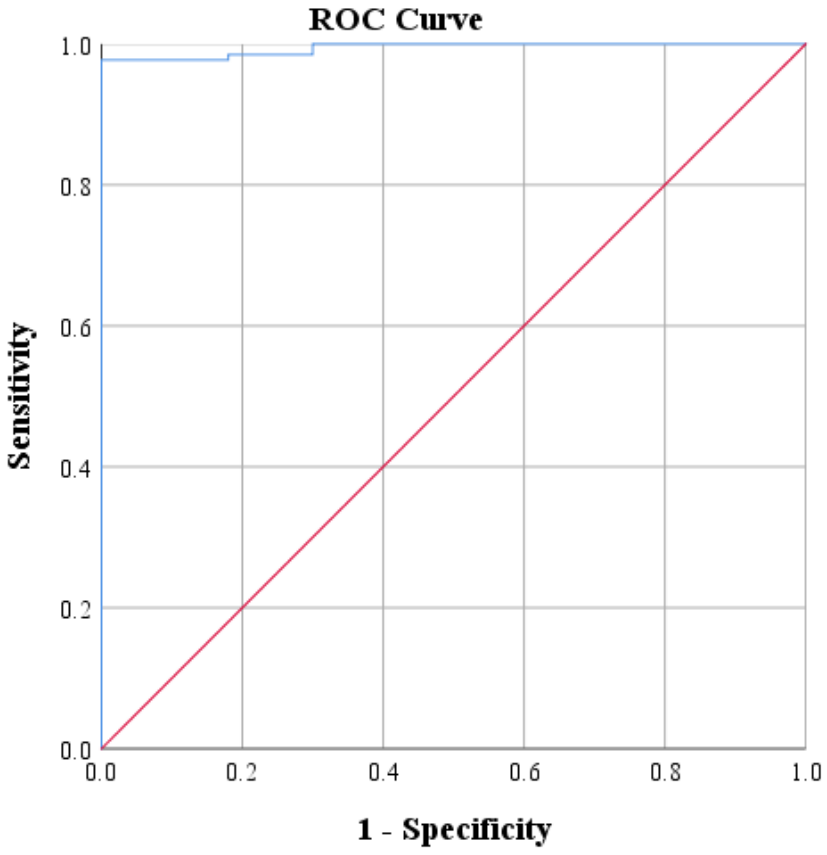


Figure 4-13: Receiver operating characteristic curve β-CTX showing sensitivity and specificity

ROC curve used for (Vit-D3, OC and OPG) and the results obtained cannot be used for discrimination between disease and healthy subjects.

The detailed result of ROC for β-CTX revealed in Table (4-13), where results are shown AUC (0.99), Sensitivity (0.99), Specificity (0.70), Cut-off points (905.78) and ($p < 0.001$).

Table 4-13: Receiver operating characteristic curve showing sensitivity and specificity of indices in lupus arthritis patients

Test Variable positive actual critical state.	AUC	Sensitivity %	Specificity %	Cut-off points	*P* vale
β-CTX	0.99	0.99	0.70	905.78	0.000

Conclusion and Recommendation

Conclusion and Recommendation

Conclusion

1- The levels OPG and β-CTX were significantly increased in lupus arthritis while OC and Vit-D3 levels were significantly decreased.

2- The OPG and β-CTX levels were of increased with disease activity while OC and Vit-D3 levels were of decreased with disease activity.

3- β-CTX showed excellent diagnostic performances for lupus arthritis where results are shown AUC (0.99), Sensitivity (0.99), Specificity (0.70), Cut-off points (905.78) and ($P < 0.001$). β-CTX was good indicator for severity of lupus arthritis.

4- The correlation of OPG with ANA, ESR, S,Cr and Vit-D3 where positive correlation while OPG with β-CTX were negative correlation. The correlation of OC with C3, Age, HB, β-CTX, OPG where positive correlation while OC with BMI was negative correlation.

5- The result of Vit-D3 were positive correlation with RBC and β-CTX, While negative correlation with C3 and HB. The result of β-CTX were positive correlation with S,Cr , BUN, ESR, WBC, RBC, OC, Ca^{+} and Vit-D3,While negative correlation with OPG and ANA.

Recommendation

1- Studying the role of vitamin K in lupus arthritis patients to understand whether it is affected by the disease because it is involved in the manufacture of OC.
2- Studying the role of β -CTX in patients with lupus nephritis and see if it is involved in kidney damage, because β -CTX is filtered by the kidneys.
3- Studying the role of Cathepsin K in lupus arthritis patients due to its association with OC inhibition.
4- According to the study's findings, it is beneficial to incorporate the BCTX examination into the standard checks conducted on female lupus arthritis patients in the laboratories of the Iraqi Ministry of Health.

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Appendix

Appendix 1

Questionnaire

Date: : :

Patient No: _____ Patient code: _____

Name:	Age:	Sex:	Social status:	Kids number :

Duration of disease:	Phone no:	Weight:	Height:

Main symptoms:

Joint pain		Arthritis	
Molar rash		Oral ulcer	
Photosensitivity		Hair lose	
Anemia		Renal failure	

Chronic diseases:

Heart disease		Diseases of the joints	
Diabetic		Pressure	
Kidney disease		Liver disease	

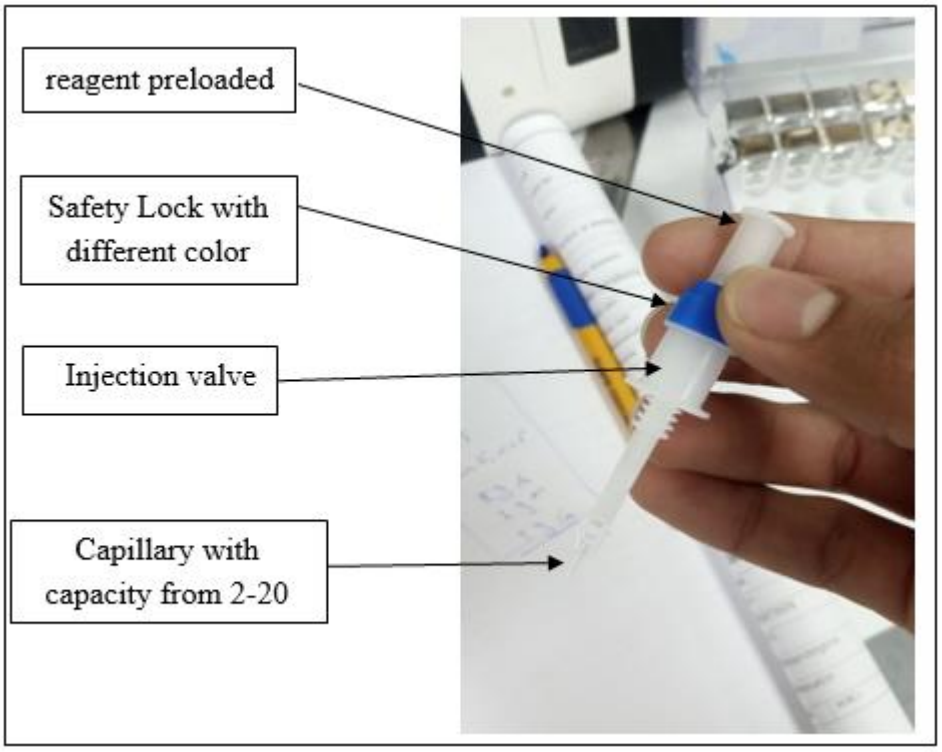
Inclusion criteria: All women with lupus arthritis, whose age ranged from 15 to 65 years, and who have been examined clinically by a specialist.

Exclusion Criteria: Patients with Tumors, Patients under 10 years old, Persons taking nutritional supplements, other inflammatory disease such as RA, Osteoporosis, Myositis vasculitis, history of hypersensitive, Treatment Cyclophosamide, chronic condition such as asthma or crohn's disease, thyroid disease and pregnant.

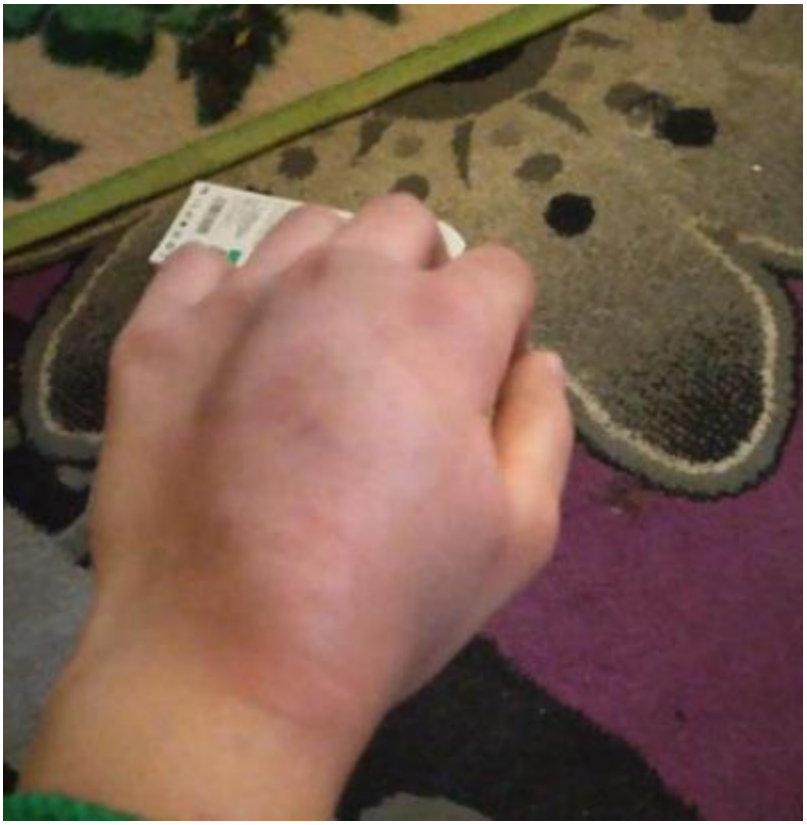
Tests

ANA		dsDNA		
Ca+		ESR		
Creatinine		C3		
Vit-D3		C4		
OPG		OC		
B (CTX)				
CBC	WBC	Hb	RBC	PLT

Appendix 2



Appendix 3



الخلاصة

التهاب المفاصل الذئبي هو التهاب الغشاء المفصلي الذي يصيب مفصلين أو أكثر ويتميز بالتورم أو الانصباب أو الألم في مفصلين أو أكثر يستمر لمدة 30 دقيقة على الأقل في الصباح. سيعاني ما يصل إلى 90٪ من مرضى الذئبة الحمراء من الإصابة العضلية الهيكلية ، مما يجعلها واحدة من أكثر أعراض الحالة انتشارا.

بحثت دراستنا في مستوى مصل OPG و BCTX و OC و Vit-D3 في المرضى المصابين بالتهاب المفاصل الذئبي. قياس تأثير شدة المرض على مستويات مصل الدم OPG و BCTX و OCوVit-D3 . لتقييم الخصوصية والحساسية ، قطع قيمة العوامل. تقييم ارتباط هذه العوامل مع العمر ، مؤشر كتلة الجسم ، ANA، dsDNA، C3 ، C4 ، CBC، ESR، S.Ca + ، BUN وS.Cr.

أجريت دراسة الحالات والشواهد هذه على 131 امرأة من مرض الذئبة الحمراء و 50 فردا سليما على مدى 4 أشهر ، من نوفمبر 2022 حتى مارس 2023. تم جمع نساء مرض الذئبة الحمراء المدرجة في دراستنا من مستشفى بغداد في المدينة الطبية ، محافظ بغداد. حدث الجزء العملي في مختبرات الأبحاث التابعة للمركز الدولي للبحوث والتطوير الكائن بمدينة الكاظمية والمختبرات التعليمية بالمدينة الطبية.

خضعت جميع نساء المصابات بداء الذئبة الاحمراري اللائي شملتهن الدراسة للفحص البدني ، والعمر ، ومؤشر كتلة الجسم ، والفجوصات المعملية ، بما في ذلك فحص البول ، و ANA ، و ds-DNA ، و BUN ، و S.Cr ، و ESR ، و dsDNA ، و C3 ، و C4 ، و+ S.Ca. تم إجراء الفحوصات السريرية اعتماداً على الطبيب الاستشاري ، وتم تصنيفها وفقًا لبرنامج Roma Helper إلى ثلاث مجموعات (غير نشطة N = 30 ، معتدلة ومتوسطة N = 49 وشديدة N = 52) وفقًا لشدة المرض.

النتائج اوضحت زيادة في مستويات OPG و β-CTX في التهاب المفاصل الذئبي بينما انخفضت مستويات OC و Vit-D3 بشكل ملحوظ. ارتبطت مستويات OPG و β-CTX ارتباطًا إيجابيًا بنشاط المرض بينما كانت مستويات OC و Vit-D3 ذات ارتباط سلبي بنشاط المرض. أظهرت β-CTX أداءً تشخيصيًا ممتازًا لالتهاب المفاصل الذئبي حيث تظهر النتائج المنطقة الواقعة تحت المنحنى (0.99) والحساسية (0.99) والنوعية (0.70) ونقاط القطع (905.78) و ($p < 0.001$). كان ارتباط OPG مع ANA و ESR و S و Cr و Vit-D3 ارتباطاً ايجابياً بينما كان OPG مع β-CTX ارتباطًا سلبيًا. وكان ارتباط OC مع C3، Age ، HB، β-CTX، OPG ارتباطاً موجبًا بينما OC مع مؤشر كتلة الجسم كان ارتباطًا سلبيًا. كانت نتيجة ارتباط D3 موجب مع RBC و β-CTX ، بينما كان هناك ارتباط سلبي مع C3 و HB. كانت نتيجة β-

CTX علاقة إيجابية مع S.Cr و BUN و ESR و WBC و RBC و OC و Ca + و Vit-D3 ، بينما الارتباط السلبي مع OPG و ANA. في الختام ، زادت مستويات OPG و β-CTX بشكل كبير في التهاب المفاصل الذئبي بينما انخفضت مستويات OC و Vit-D3 بشكل ملحوظ.

في المحصلة ضهرت β-CTX مؤشرًا جيدًا لشدة التهاب المفاصل الذئبي.

جامعة كربلاء

كلية العلوم الطبية التطبيقية

قسم التحليلات المرضية

دراسة بعض المعلمات المناعية و الكيموحيوية في المصابات بالتهاب المفاصل الذئبي

الرسالة مقدمة

إلى مجلس كلية العلوم الطبية التطبيقية - جامعة كربلاء

وهي جزء من متطلبات نيل

شهادة الماجستير في التحليلات المرضية

كُتيبت بواسطة

(محمد ربيع ناصح)

بكالوريوس تقنيات التحليلات المرضية / كلية المصطفى الجامعة 2018

بأشراف

أ.د. حسن علي حسين **أ.م د. رضوان محمد حسين**

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